

Transport Phenomena in Pomegranate Fruit: Mechanisms of Weight Loss and Control Strategies

by

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Declaration

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Abstract

The revived interest in this ancient fruit Pomegranate (*Punica grantum* L) has resulted in increased production, consumption and intensified research owing to its health and nutritional benefits. Pomegranates are considered luxurious fruit that sell well in the higher market segment and there has been a growing demand for high quality, healthy and exotic fruit both for fresh use and for processing into juices and other products. However, the fruit is classified as highly perishable and specifically being prone to moisture loss, irrespective of its thick rind and tough leathery outer skin. Water loss can easily cause a huge financial loss to the industry through direct loss of marketable fresh weight and the associated diminished commercial value of affected fruit. Therefore, the overall aim of this research was to investigate the mechanisms of weight loss in pomegranate fruit by characterising associated structural and quality changes in the fruit, quantifying the water transport properties of fruit tissues, developing a water transport model and to assess various techniques to control fruit water loss. The research methodology followed a multifaceted approach which included the application of imaging and computational techniques in combination with several laboratory experiments.

The experimental studies established that ‘Wonderful’ and ‘Herskawitz’ pomegranate cultivars are more susceptible to water loss than ‘Acco’ due to differences in aril-peel ratio and moisture content. The thick non-edible peel (rind) is the major source of water loss compared to the edible portion (arils). Overall, mean fruit water loss was 0.31, 0.34 and 0.26 g cm⁻² for ‘Wonderful’, ‘Herskawitz’ and ‘Acco’, respectively, after 16 d in shelf storage at 23 °C and 58 % relative humidity (RH). Analysis of fruit micro-structures showed that the arils (edible portion) are protected against excessive moistures loss by the inner epidermis membrane (white membrane) covering the aril sacs. This membrane had a lower water permeability (0.14×10^{-11} kg m⁻¹ pa⁻¹ s⁻¹) compared to the exocarp ($1.31\text{-}1.43 \times 10^{-11}$ kg m⁻¹ pa⁻¹ s⁻¹) and mesocarp ($13.51\text{-}16.37 \times 10^{-11}$ kg m⁻¹ pa⁻¹ s⁻¹) peel tissues under ambient conditions (23 °C and 58 % RH). Transpiration is the major process of water loss in pomegranate fruit; however, this study showed that water loss due to the respiratory process contributed up to 35 at high 93 % RH.

The qualitative studies on fruit peel microstructure using scanning electron microscopy (SEM) identified a higher count of lenticels, larger lenticel size and a generally low peel thickness on the top and mid spatial locations along the fruit surface as compared to the bottom locations. Furthermore, X-ray examination revealed higher porosity in the exocarp than in the mesocarp peel fractions and that porosity increased from bottom to middle to top locations in

the exocarp fraction. This suggests that the pomegranate fruit is more susceptible to moisture loss at the top and mid locations compared to the bottom location. In addition, a distinctively bright waxy cuticle was identified on the surface of the peel using confocal laser scanning microscopy (CLSM). A decreasing profile of the waxy cuticle thickness, increased fragmentation of waxy cuticle, widening and deepening of micro-cracks and general decrease in peel thickness were observed during fruit storage. These micro-structural features predispose the fruit to increasing water loss during storage.

The study has also demonstrated the use of non-invasive technology such as magnetic resonance imaging (MRI) to assess transient water profiles across intact fruit. Furthermore, the study developed and validated a water transport model that can be used to study water loss and control strategies in future. Assessing fruit weight loss control strategies proved that surface waxing is a potential environmentally friendly solution for minimising water loss in pomegranates. However, modelling of gas transport is required for accurate optimisation of surface waxing application. Future research should also consider coupling of water transport model and mechanical deformation model to account for shrinking (also referred to as shrivelling, wilting) of the fruit due to moisture loss.

Opsomming

Die herleefde belangstelling in hierdie antieke vrugtegranaat (*Punica grantum* L) het gelei tot verhoogde produksie, verbruik en intensiewe navorsing weens die gesondheids- en voedingsvoordele daarvan. Granate word beskou as luukse vrugte wat goed verkoop in die hoër marksegment en daar is 'n groeiende vraag na gesonde en eksotiese vrugte van hoë gehalte, sowel vir vars gebruik as vir verwerking van sappe en ander produkte. Die vrugte word egter as baie bederfbaar geklassifiseer en is spesifiek geneig tot vogverlies, ongeag die dik skil en taai leeragtige buitenste vel. Waterverlies kan maklik tot 'n groot finansiële verlies lei vir die bedryf, deur direkte verlies aan bemarkbare vars gewig en die gepaardgaande verminderde kommersiële waarde van die geaffekteerde vrugte. Die primêre doel van die navorsing was gevolglik om die meganismes van gewigsverlies by granaatvrugte te ondersoek deur gepaardgaande strukturele en kwaliteitveranderinge in die vrugte te karakteriseer, die watervervoer eienskappe van vrugweefsel te kwantifiseer, 'n watervervoermodel te ontwikkel, en waterverliesbeheertegnieke toe te pas en assesser. Die navorsingsmetodiek het 'n veelvlakkige benadering gevolg deur beeldmodaliteite en rekenaartegnieke toe te pas gepaard met velerlei laboratorium eksperimente.

Die eksperimentele studies het vasgestel dat kultivars 'Wonderful' en 'Herskawitz' meer vatbaar is vir waterverlies as 'Acco' as gevolg van verskille in die skil-verhouding en voginhoud. Die dik nie-eetbare skil is die primêre bron van waterverlies in vergelyking met die eetbare gedeelte (arils). Die algehele gemiddelde waterverlies was 0.31, 0.34 en 0.26 g cm⁻² respektiewelik vir 'Wonderfull', 'Herskawitz' en 'Acco' na 16 d in rakberging teen 23 °C en 58 % relatiewe humiditeit (RH). Analise van die vrug se mikrostruktuur dui dat die arils (eetbare gedeelte) beskerm word teen oormatige waterverlies deur die inwendige epidermis membraan (wit membraan) wat die aril beskermhulsels bedek. Hierdie membraan het 'n laer waterdeurlaatbaarheid ($0.14 \times 10^{11} \text{ kg m}^{-1} \text{ pa}^{-1} \text{ s}^{-1}$) in vergelyking met die epikarp ($1.31\text{-}1.43 \times 10^{11} \text{ kg m}^{-1} \text{ pa}^{-1} \text{ s}^{-1}$) en mesokarp ($13.51\text{-}16.37 \times 10^{11} \text{ kg m}^{-1} \text{ pa}^{-1} \text{ s}^{-1}$) skilweefsels onder ambiënt toestande (23 °C en 58 % RH). Transpirasie is die primêre proses van waterverlies by granaatvrugte; hierdie studie dui egter aan dat die asemhalingsproses bydra tot die verlies van vrugte tot by 35 teen hoë 93 % RH.

Die kwalitatiewe studies oor die skilmikrostruktuur, met behulp van skanderingselektronmikroskopie (SEM), het 'n hoër aantal lentiselle, groter lentiselgrootte en 'n algemeen lae skildikte op die boonste en middelste ruimtelike oppervlakareas van die

vrugsfeer geïdentifiseer, in vergelyking met die onderste oppervlakareas van die vrug. Verder het

'n X-straalondersoek 'n hoër porositeit in die epikarp geopenbaar as in die skilfraksies van die mesokarp en dat die poreusheid van onder na middel na bo in die epikarp-fraksie toeneem het. Dit dui daarop dat die granaatvrugte meer vatbaar was vir vogverlies op die boonste en middelste oppervlakareas in vergelyking met die onderste oppervlakarea. Daarbenewens is 'n kenmerkende helder, wasagtige kutikula op die oppervlak van die skil geïdentifiseer met behulp van konfokale laser skandeer-mikroskopie (CLSM). 'n Afnemende profiel van die wasagtige kutikula-dikte, 'n verhoogde fragmentasie van die wasagtige kutikula, die verbreding en verdieping van die mikro-krake en 'n algehele afname in die dikte van die skil is tydens die opberging van vrugte waargeneem.

Die studie het ook die gebruik van nie-indringende tegnologie, soos magnetiese resonansie beelding (MRI), gedemonstreer om die kortstondige waterprofile oor ongeskonde vrugte te assesser. Verder het die studie 'n watervervoer model ontwikkel, gevalideer en suksesvol gebruik in die voorspelling van waterverlies van krimp toegedraaide, voering verpakte, gewas en ongewasde granaatvrugte. Hierdie model kan in die toekoms gebruik word om waterverlies en beheerstrategieë te bestudeer. Die studie beklemtoon oppervlakwas as 'n moontlike omgewingsvriendelike oplossing vir die vermindering van waterverlies in die vrugte. Modellerings van gasvervoer is egter nodig vir die akkurate optimalisering van die toediening van die oppervlakwas. Toekomstige navorsing kan ook die koppeling van die watervervoermodel en meganiese vervormingsmodel oorweeg om die inkrimping van die vrug as gevolg van vogverlies te verantwoord.

This thesis is dedicated to my lovely wife Mrs Lesley-Ann Lufu and daughter Shiloh Shalom Lufu

Even perfection has its limits, but God's commands have no limit. Mem (Psalms 119:9 NLT)

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Preface

This thesis is a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable. Language and styles used in this thesis are in accordance with the requirements of the International Journal of Food Science and Technology.

Table of contents

Declaration	i
Abstract	ii
Opsomming	iv
Acknowledgement	vii
Preface	ix
Table of contents	x
1 General introduction	1
1.1 Background	1
1.2 Research aim and objectives	4
1.3 Thesis structure.....	4
2 Water loss of fresh fruit: influencing pre-harvest, harvest and postharvest factors	12
2.1 Introduction	12
2.2 Pre-harvest factors.....	15
2.2.1 Produce type	15
2.2.2 Orchard practices.....	21
2.3 Harvest factors	25
2.3.1 Fruit maturity	25
2.3.2 Weather condition and climatic season	25
2.3.3 Harvest technique and physical injury	26
2.3.4 Tree age and canopy position	27
2.4 Postharvest factors	27
2.4.1 Temperature	27
2.4.2 Relative humidity	28
2.4.3 Storage duration	30
2.4.4 Airflow.....	31
2.4.5 Packaging	32
2.4.6 Surface coating.....	34
2.4.7 Other pre-treatments	36
2.5 Conclusion and prospects	38
3 A review of the mechanisms and modelling approaches to water loss in fresh fruit	55
3.1 Introduction	55
3.2 Physiological mechanisms of water loss	57
3.2.1 Transpiration	57
3.2.2 Respiration	58
3.2.3 Other mass loss mechanisms	60

3.3	Product structure-water loss relations	61
3.3.1	Macro-structure	61
3.3.2	Micro-structure	62
3.4	Modelling approach to moisture transport	64
3.4.1	Levels of scale	64
3.4.2	Modelling approaches	70
3.5	The role of imaging technologies.....	71
3.6	Conclusion.....	73
4	Characterising water loss and quality attributes of pomegranate fruit cultivars ('Acco', 'Herskawitz' & 'Wonderful') under cold and shelf storage conditions.....	85
4.1	Introduction	86
4.2	Materials and methods.....	87
4.2.1	Fruit acquisition	87
4.2.2	Experimental design.....	88
4.2.3	Measurements	89
4.2.4	Calculations	91
4.2.5	Statistical analysis	93
4.3	Results and discussion	93
4.3.1	Fruit size and water loss.....	93
4.3.2	Respiration rate (<i>RR</i>).....	96
4.3.3	Peel colour of the three cultivars.....	98
4.3.4	Fruit firmness	101
4.3.5	Physical and chemical characteristics of fruit fractions.....	103
4.3.6	Multivariate analysis	110
4.4	Conclusion.....	115
5	The contribution of transpiration and respiration processes in the mass loss of pomegranate fruit (cv. Wonderful)	122
5.1	Introduction	122
5.2	Materials and methods.....	124
5.2.1	Fruit sample	124
5.2.2	Experimental setup	125
5.2.3	Measurement procedures	126
5.2.4	Calculations	128
5.2.5	Statistics	131
5.3	Results and discussion	131
5.3.1	Mass loss and transpiration rate.....	131

5.3.2	Respiration rate.....	136
5.3.3	Water activity.....	138
5.3.4	The contribution of respiration and transpiration to overall mass loss	138
5.4	Conclusions	142
6	Determining water transport properties (diffusivity and permeability) of pomegranate fruit tissues	150
6.1	Introduction	150
6.2	Materials and methods	152
6.2.1	Fruit acquisition	152
6.2.2	Experimental design.....	152
6.2.3	Sample preparation	153
6.2.4	Moisture diffusion experimental set up	154
6.2.5	Calculation of water transport properties	155
6.2.6	Statistics	155
6.3	Results and discussion	156
6.3.1	Analysis of variance.....	156
6.3.2	Effect of tissue type	156
6.3.3	Effect of tissue location on the fruit	162
6.3.4	Effect of storage duration	163
6.3.5	Effect of temperature of determination.....	163
6.4	Conclusion.....	164
7	Identification and characterisation of the peel surface structures of pomegranate fruit (cv. Wonderful) during storage	171
7.1	Introduction	171
7.2	Materials and methods	173
7.2.1	Fruit acquisition	173
7.2.2	Experimental design.....	174
7.2.3	Sample preparation	174
7.2.4	Qualitative analysis	176
7.2.5	Quantitative measurements and calculations	176
7.3	Results and discussion	178
7.3.1	Identification of surface structures with SEM	178
7.3.2	Changes in peel surface structures	182
7.3.3	Identification of peel layers with CLSM	194
7.3.4	Changes in waxy cuticle thickness	199
7.3.5	Changes in macro peel fraction thickness	201

7.4	Conclusion.....	203
8	Analysing changes in the 3D microstructural pore space of pomegranate peel fractions with fruit weight loss using X-ray micro-CT	210
8.1	Introduction	210
8.2	Materials and methods.....	212
8.2.1	Fruit acquisition	212
8.2.2	Experimental design.....	212
8.2.3	Weight loss monitoring.....	212
8.2.4	Sample preparation before scanning.....	213
8.2.5	X-ray micro-computed tomography imaging.....	214
8.2.6	Image processing	215
8.2.7	Pore space analysis	215
8.3	Results and discussions.....	216
8.3.1	Fruit weight loss.....	216
8.3.2	Pore space in peel fractions	217
8.3.3	Pore space distribution with location on fruit	220
8.3.4	Changes in pore space during fruit storage	222
8.3.5	Pore space characteristics.....	223
8.4	Conclusion.....	227
9	Computational modelling of the moisture transport in pomegranate fruit under cold storage and shelf life conditions— part I: measuring the moisture transport parameters	235
9.1	Introduction	235
9.2	Materials and methods.....	238
9.2.1	Pomegranate fruit sample	238
9.2.2	Sample preparation	238
9.2.3	X-ray computed tomography (CT) scanner.....	239
9.2.4	Image analysis and statistics	240
9.2.5	Modelling the moisture transport using Lattice-Boltzmann method (LBM)	241
9.3	Results and discussions.....	244
9.3.1	Analysis of the pore network.....	244
9.3.2	The 3D transient water transport in the peel samples.....	247
9.4	Conclusion.....	249
10	Computational modelling of the moisture transport in pomegranate fruit under cold storage and shelf life conditions— part 2: finite volume model development and validation using magnetic resonance imaging.....	256
10.1	Introduction	256
10.2	Materials and methods.....	258

10.2.1	Fruit acquisition	258
10.2.2	Water diffusion model	258
10.2.3	Model validation	260
10.3	Results and discussion	265
10.3.1	Simulation of 3D water distribution.....	265
10.3.2	Validation of 3D water distribution	267
10.3.3	Transient water profiles	269
10.4	Conclusions	270
11	Assessing weight loss control strategies in pomegranate (<i>Punica granatum</i> L.) fruit.....	275
11.1	Introduction	275
11.2	Materials and methods.....	277
11.2.1	Fruit acquisition	277
1.1.1	Packaging and waxing	277
11.2.2	Fruit storage.....	278
11.2.3	Measurements and evaluation	278
11.2.4	Calculations	280
11.2.5	Statistical analysis	281
11.3	Results and discussion	282
11.3.1	Weight loss.....	282
11.3.2	Total colour difference (TPC) of the peel.....	284
11.3.3	Respiration rate (<i>RR</i>).....	287
11.3.4	Respiratory quotient (<i>RQ</i>).....	289
11.3.5	TSS and TA.....	291
11.3.6	Fruit decay incidence	294
11.3.7	Sensory evaluation.....	295
11.3.8	SEM	296
11.4	Conclusion.....	297
12	General discussion and conclusions.....	305
1.2	Introduction	305
1.3	General discussion	305
1.4	General conclusion and feature prospects	314
1.5	References	315

List of published and submitted articles, and conference presentations from this thesis

- Lufu, R., Berry, T.M., Ambaw, A. & Opara, U.L. (2018). The influence of liner packaging on weight loss and decay of pomegranate fruit. *Acta Horticulturae*, **1201**, 259–264.
- Lufu, R., Ambaw, A. & Opara, U.L. (2019). The contribution of transpiration and respiration processes in the mass loss of pomegranate fruit (cv. Wonderful). *Postharvest Biology and Technology*, **157**, 110982.
- Lufu, R., Ambaw, A. & Opara, U.L. Characterising weight loss and quality attributes of pomegranate fruit cultivars (‘Acco’, ‘Herskowitz’ & ‘Wonderful’) under cold and shelf storage conditions. *Journal of Food Measurement and Characterization*. Under review.
- Lufu, R., Ambaw, A. & Opara, U.L. A review on the loss of water in fresh fruit: influencing pre-harvest, harvest and postharvest factors. *Scientia Horticulturae*. Under review.

SECTION I

General introduction, aim & objectives and a review of literature on the factors influencing postharvest water loss, underlying mechanisms and the modelling approach on water loss (Chapter 1-3)

CHAPTER 1

GENERAL INTRODUCTION

Chapter 1

1 General introduction

1.1 Background

Pomegranate (*Punica granatum* L.) fruit is a berry belonging to the family Lythraceae. The fruit is comprised of an edible portion (arils) which accounts for about 40-65 % and non-edible portion (often referred to as rind or peel) which accounts for about 35-60 % of whole fruit mass (Al-Said *et al.*, 2009; Wetzstein *et al.*, 2011; Fawole and Opara, 2013a). The fruit is often eaten fresh, processed into other products such as juice, wine and jam (Kader, 2006; Opara *et al.*, 2009) or used as raw material in the cosmetic and pharmaceutical industries. Iran is the leading producer of pomegranate fruit, producing over one million tonnes on about 70, 000 ha of commercial land (IMA, 2016). South Africa pomegranate production is estimated at 7,337 tonnes per annum, with 69 % of the fruit exported (NAMC, 2017) and a commercial growing area of about 932 ha (Hortgro, 2018). Exports have increased over the years from 1,877.7 tonnes in 2012 to 5,475.0 tonnes in 2017 (Hortgro, 2018). Freshly harvested fruit is kept under cold storage awaiting export to distant markets. Fruit from South Africa takes a maximum period of about 6 weeks to reach Europe, which is the major export market destination. Storing pomegranate ‘Wonderful’ for 12 weeks at 5 °C and > 92 % RH minimises physiological disorders, maintains internal and external quality attributes (Arendse *et al.*, 2014).

In postharvest handling of pomegranate fruit, weight loss is a major problem besides other physiological disorders like fruit decay, chilling injury, scalding and browning, contributing to quantitative and qualitative loss (Caleb *et al.*, 2012; Valero *et al.*, 2015). The fruit has an average fresh weight water content of about 74.1-89.6 % (Hellen *et al.*, 2014; Mukama *et al.*, 2018), which centrally influences water transport and the postharvest quality of the fruit. Transpiration and respiration processes continue after harvest and deplete water and carbohydrate substrates, respectively, causing produce weight loss through moisture and carbon dioxide diffusion and heat dissipation to the atmosphere (Maguire *et al.*, 2000; Ben-Yehoshua and Rodov, 2003). Weight loss is commonly through transpiration (moisture loss) process than through depletion of respiration substrates as observed in pear fruit ‘Kontoula’ (Xanthopolous *et al.*, 2017). Transpiration can contribute as high as 97 % to total weight loss in tomatoes (Shirazi and Cameron, 1993). However, under conditions of high relative humidity and temperature, respiration contributes greatly to weight loss (Xanthopolous *et al.*, 2017).

Fresh produce is susceptible to losing moisture through open stomata and or lenticels, micro-cracks, damaged tissues, scars of detachment like the calyx and across the cuticle (Maguire *et al.*, 1999; Knoche and Peschel, 2007; Ben-Yehoshua and Rodov, 2003; Theron, 2015). Specifically, pomegranates are highly prone to moisture loss owing to the relatively high water permeability across the skin through minute slits, despite having a thick rind (Nanda *et al.*, 2001; Opara *et al.*, 2010). Research conducted in South Africa showed that cultivars such as ‘Bhagwa’, ‘Ruby’ and ‘Wonderful’ can lose 20-25 % of initial fruit weight within 4 weeks under ambient storage of about 22 °C and 65 % RH (Fawole and Opara, 2013b; Arendse *et al.*, 2014). During prolonged cold storage, the fruit ‘Bhagwa’, ‘Ruby’ and ‘Wonderful’ lose between 10-16 % of their weight within 12 weeks at 5-7 °C and 90-95 % RH (Fawole and Opara, 2013b; Arendse *et al.*, 2014; Mphahlele *et al.*, 2016; Lufu *et al.*, 2018). In comparison, ‘Star Ruby’ grapefruit lost about 4.2 % of their weight when stored at 10 °C and 80-85 % RH for 16 weeks plus additional 1 week at 20 °C (Chaudhary *et al.*, 2015). ‘Bravo de Esmolfe’ apples lost 7 % in weight at 2 °C and 85 % RH for 26 weeks (Rocha *et al.*, 2004). It is important to note that fruit are commonly sold on fresh weight basis; therefore weight loss in fruit represents considerable economic loss to the industry.

Pomegranates are considered luxurious fruit that sell well in the higher market segment (CBI, 2019). Therefore water loss can easily cause a huge financial loss to the industry through direct loss of marketable fresh weight and the associated diminished commercial value of affected fruit (Pathare *et al.*, 2013). Generally, excessive moisture loss may result into browning, loss in fruit texture, firmness and flavour of fresh fruit (Ben-Yehoshua and Rodov, 2003; Vigneault *et al.*, 2009; Caleb *et al.*, 2013), as well as promoting accelerated senescence, susceptibility to chilling injury and membrane disintegration (Kays and Paull, 2004). Specifically, in pomegranates, rind hardening, rind and aril browning are expected due to excessive moisture loss (Artés *et al.*, 2000; D’Aquino *et al.*, 2010). Literature reports that a 5 % weight loss is enough to initiate shrivelling in fresh fruit (Mitchell and Kader, 1989) because of loss in turgidity of the peel cells.

To improve the understanding of weight loss in fresh commercial fruit during storage, studies have been conducted on transpiration and moisture permeability across the cuticles and waxy layers (Veraverbeke *et al.*, 2003). As a result, weight loss and transpiration models have been developed to describe and predict weight loss in harvested fruit. The developed models can be categorised as empirical, semi-empirical and analytical models (Xanthopoulos *et al.*, 2017). In addition, microscopic studies have been carried out to establish a good understanding

of the influence of fruit structure on water loss. In this regard, studies using conventional light microscope have revealed presence and quantity of lenticels on the fruit peel. Scanning electron microscope (SEM) and confocal scanning microscope (CSM) have showed the existence of micro-cracks on the peels of different apple cultivars (Maguire *et al.*, 2001; Martynenko, 2011, Veraverbeke *et al.*, 2003). Furthermore, the transient transport of water across different membranes of the fruit has been studied using magnetic resonance imaging (MRI) (Nguyen *et al.*, 2006). This technique enables non-destructive mapping of moisture concentration inside the fruit (Verstreken *et al.*, 1998; Burdon and Clark, 2001; Paniagua *et al.* 2013).

Many techniques have been applied in the fresh fruit industry to minimise moisture loss, including cold chain management, intermittent warming, surface coating and waxing (Park *et al.*, 1994; Nanda *et al.*, 2001). Heat shrinkable wrapping is reported to significantly minimise water loss in pomegranate fruit (Artés *et al.*, 2000; Nanda *et al.*, 2001; D'Aquino *et al.*, 2010). However, shrink wrapping and surface coating / waxing can lead to anaerobic respiration of the fruit by creating an oxygen deficit and high CO₂ atmosphere for the fruit. This results in production of off-flavours and undesirable changes in fruit taste (Gil *et al.*, 1996; Canti'n *et al.*, 2008). Modified atmosphere packaging (MAP) of fruit in plastic liners is a widely applied technique on various fruit. However, the use of liners may give rise to moisture condensation within the bags and around the fruit promoting fruit decay (Wiley *et al.*, 1999; Lufu *et al.*, 2018). Furthermore, the use of plastic liners is limited for use during cold storage only and not applicable during shelf market conditions. Therefore, there is a need for optimised strategic control techniques. Optimising existing and developing new control strategies requires focussed scientific knowledge of the water transport phenomena in fresh fruit. However, the underlying mechanisms of weight loss in pomegranate fruit are quite unclear.

Understanding the mechanisms of fruit weight loss in pomegranates is of particular interest among researchers within the community for several reasons: the fruit is prone to high weight loss even under cold chain conditions, compared with other type of fruit; the fruit structure involving a thick non-edible rind (40-48 %) surrounding the edible portion (Arils) which contains > 80 % water; the weight loss mechanisms of other commonly studied fruit like pears and apples cannot easily be extrapolated to include pomegranates due to biological and structural differences.

1.2 Research aim and objectives

The aim of this research was to investigate the mechanisms of weight loss in pomegranate fruit as a foundation for strategic control techniques by applying imaging and computational methods. The research aim was accomplished through the following specific objectives:

1. To assess weight loss susceptibility of commercial pomegranate fruit cultivars ('Acco', 'Herskawitz' and 'Wonderful');
2. To investigate the contribution of transpiration and respiration mechanisms to weight loss of pomegranate fruit (cv. Wonderful) during storage;
3. To characterise changes in the peel structure of pomegranate fruit (cv. Wonderful) due to weight loss during storage, using X-ray computed tomography and scanning microscopy imaging;
4. To assess water transport properties of pomegranate fruit (cv. Wonderful) at tissue level;
5. To develop a water transport model for pomegranate fruit (cv. Wonderful) using finite volume computational analysis and model validation using magnetic resonance imaging (MRI); and
6. To assess weight loss control strategies during postharvest handling of pomegranate fruit (cv. Wonderful).

1.3 Thesis structure

This dissertation was structured into five sections (I-V), with each section addressing a research theme and comprising of two-three chapters. The individual chapters are manuscripts published, submitted or prepared for publication.

Section I: Presents general introduction, research aim and objectives as well as review of literature on the factors influencing postharvest water loss in fresh fruit, underlying mechanisms of weight loss and the modelling approach on water loss (Chapter 1-3).

Section II: Focusses on quantitative laboratory experiments to investigate water loss patterns in pomegranate fruit cultivars, the contribution of transpiration and respiration processes to fruit mass loss and determining water transport properties of pomegranate fruit tissues (Chapter 4-6).

Section III: Reports on qualitative examination of the microstructure of pomegranate peel using scanning electron microscopy, confocal laser scanning microscopy and x-ray micro computed tomography (Chapter 7 & 8).

Section IV: Focusses on modelling water transport in pomegranate fruit and model validation. Secondly, water loss control strategies were assessed (Chapter 9-11).

Section V: Presents a general discussion integrating the results from all the previous chapters and highlights practical contribution of the studies to the pomegranate industry (Chapter 12).

Each review or research chapter has been written in a form suitable for journal publication. Therefore, some repetition may be necessary.

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CHAPTER 2

A REVIEW ON THE LOSS OF WATER IN FRESH FRUIT: INFLUENCING PRE-HARVEST, HARVEST AND POSTHARVEST FACTORS

Declaration by the candidate

With regard to Chapter 2, pages 12–52, the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Literature search and writing of chapter	80

The following co-authors have contributed to Chapter 2, pages 12–52:

Name	e-mail address	Nature of contribution	Extent of contribution (%)
Alemayehu Ambaw	tsige@sun.ac.za	Research input, editorial suggestion and proof reading	10
Umezuruike Linus Opara	opara@sun.ac.za	Conceptualised the review and and edited the document	10

Declaration with signature in possession of candidate and supervisor	26/02/2020
Signature of candidate	Date

Declaration by co-authors

The undersigned hereby confirm that:

1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 2, pages 12–52,
2. no other authors contributed to Chapter 2, pages 12–52 besides those specified above, and
3. potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 2, pages 12–52 of this dissertation.

Signature	Institutional affiliation	Date
Declaration with signature in possession of candidate and supervisor	Department of Horticultural Sciences, Stellenbosch University	26/02/2020
Declaration with signature in possession of candidate and supervisor	Department of Horticultural Sciences, Stellenbosch University	26/02/2020

Chapter 2

2 Water loss of fresh fruit: influencing pre-harvest, harvest and postharvest factors

Abstract

Physiological water loss is one of the many postharvest disorders in the fresh fruit industry. Water loss initiates wilting, shrivelling, browning, loss in fruit texture, flavour, and saleable weight and accelerates senescence. A clear understanding of the factors influencing water loss is crucial in optimising the control strategies. This knowledge is also required to design and operate storage facilities to ensure the extension of the shelf life of fresh fruit and vegetables. This paper identifies, interprets and discusses the major research works and findings relating to the pre-harvest, harvest and postharvest factors influencing the water loss in fresh fruit. The review acknowledges that postharvest water loss varies greatly among fresh produce given the multitude of factors discussed in this review. The environmental factors (temperature and humidity) have a strong influence on fruit water loss. The rate of water loss also differs among species and even among cultivars of the same species as this determines the fruit factors (the fruit surface-area-to-volume ratio, the surface structure of the fruit, including the number and size of stomata and lenticels, and the thickness and composition of the cuticle). Currently, the water loss characteristics of many commercially important fruit are not adequately studied; therefore, a knowledge gap exists in understanding their mechanisms of losing water. Yet the large biological difference among fruit types makes it difficult to extrapolate such knowledge. This study systematically reviewed literature related to water loss in the pre-harvest, harvest and postharvest management of commercially important fruit.

2.1 Introduction

The nutritional and health benefits of consuming fresh fruit is scientifically well proven. Fruit and vegetables are rich in dietary fibre, phytochemicals and micronutrients and are associated with minimising the risk of cardiovascular diseases and gastrointestinal cancers (Yahia, 2009; Opara and Al-Ani, 2010; Hussein *et al.*, 2015). It is recommended to consume various fruit and vegetables in adequate amounts to reach the minimum daily recommended level of 400 g (WHO, 2003). Currently, the demand for fresh fruit is increasing due to a growing awareness about nutrition and healthy lifestyles (Caleb *et al.*, 2013c; Li and Thomas, 2014).

Fruit and vegetables are highly perishable, challenging the effort to meet the ever-increasing demand. The supply chain worldwide is characterised by a high rate of losses, with the highest losses occurring between harvest and consumption. About half (45 to 55 %) of the global fruit and vegetable production is lost or wasted along the value chain from production to consumption as compared to 35, 30 and 20 % for seafood, cereals and meats, respectively (FAO, 2011; Lipinski *et al.*, 2013). Fruit and vegetables are prone to water loss, which is a major cause of postharvest deterioration and quality downgrade (Kays and Paull, 2004; Nunes and Emond, 2007). Fruit water loss (Hatfield and Knee, 1988; Harker *et al.*, 2019) is often used interchangeably as weight loss (Maguire *et al.*, 2010; Lufu *et al.*, 2018), mass loss (Paull *et al.*, 1997; Maguire *et al.*, 1999) and moisture loss (Maw and Mullinix, 2005; Ngcobo *et al.*, 2012; Paniagua *et al.*, 2013). In this current work, water loss will be adopted.

Most fruit and vegetables become unmarketable with a water loss of 5 % to 10 % of their initial weight (Robinson *et al.*, 1975) as this leads to quality loss by initiating wilting and shrivelling (Ben-Yehoshua and Rodov, 2002). Excessive water loss, in addition to causing loss of saleable weight, promotes browning, loss in fruit texture and flavour (Ben-Yehoshua and Rodov, 2002; Vigneault *et al.*, 2009; Caleb *et al.*, 2013a), accelerated senescence, susceptibility to chilling injury and membrane disintegration (Ben-Yehoshua, 1983; Kays and Paull, 2004).

Although the direct and indirect impact of water loss on the quality of harvested produce is quite obvious, the mechanism of water loss is a complex process that is affected by many factors involving the physiological, biochemical and physio-mechanical properties of the produce as well as the production, harvest and postharvest treatments and handling (**Figure 2.1**). Over the last decades, most research studies have focused on investigating the postharvest factors neglecting the pre-harvest and harvest factors. Recent studies have shown that pre-harvest and harvest factors substantially contribute to postharvest water loss (Elshiekh and Abu-Goukh, 2008; Laribi *et al.*, 2013; Peña *et al.*, 2013; Lobos *et al.*, 2016; Tyagi *et al.*, 2017). The daily orchard management practices, climate and seasonal variations influence fresh produce quality after harvest to a great extent (Opara, 2007; Tahir *et al.*, 2007; Prusky, 2011). There is currently limited knowledge on the influence of the pre-harvest and harvest factors on the water loss of fresh produce during postharvest handling. Secondly, the existing knowledge on the postharvest factors influencing fruit water loss during storage requires consolidation to facilitate the selection of an appropriate combination of control strategies. Despite vast research on water loss control technologies and preservation of postharvest quality, there is a lack of integrated review on the pre-harvest, harvest and postharvest factors influencing water loss

during storage conditions. Understanding of the multi-stage and multi-level factors affecting water loss in the fresh fruit supply chain will give new insight into new areas of research. Hence, the paper provides an extensive review of the pre-harvest, harvest and postharvest factors influencing the postharvest water loss of fresh fruit. This knowledge is relevant in aiding the selection and design of optimum conditions along the value chain, to ensure an extended shelf life of fresh fruit.

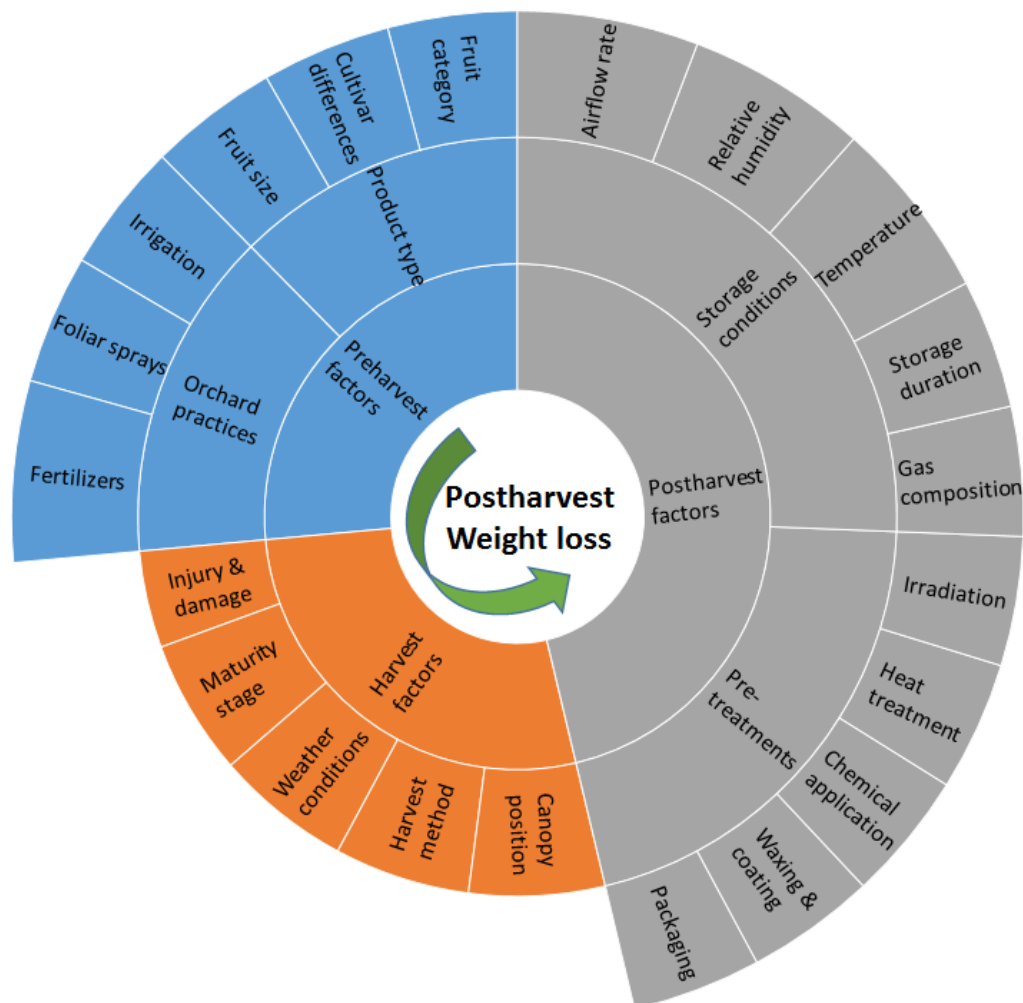


Figure 2.1 Sunburst chart showing the pre-harvest, harvest and postharvest factors influencing postharvest water loss of fresh produce.

2.2 Pre-harvest factors

2.2.1 Produce type

2.2.1.1 Fruit category

Table 2.1 shows the rate of water loss for different fresh fruit types during low, medium and high temperature storage conditions. Closely related fruit are observed to have comparable rates of water loss. For example, climacteric drupes such as peach and nectarine have a comparably similar water loss rate of 0.77 and 0.74 % d⁻¹, respectively during low temperature (2 °C and 90 % RH) storage (Serrano *et al.*, 2004). Quite similar results were observed between peach and plum under shelf storage at 20 °C and 85 % RH (Guillén *et al.*, 2013). This can be attributed to similar anatomical and morphological structuring in stone fruit. A similar characteristic arrangement of successive pericarp layers has been observed in plum, peach, apricot and nectarines (Archibald and Melton, 1987; King *et al.*, 1987; Konarska, 2015). However, a relatively lower rate (0.17-0.31 % d⁻¹) than for peach and nectarine is observed in plums under similar storage conditions (Serrano *et al.*, 2004; Valero *et al.*, 2013).

In climacteric pome fruit, a higher rate of water loss is observed in pears (0.24 % d⁻¹) than in apples (0.06 % d⁻¹) under similar shelf storage conditions of 20 °C and 95 % RH (Tu *et al.*, 2000; Xanthopoulos *et al.*, 2017). A similar situation was observed between apples and pears under relatively similar cold storage conditions (Rocha *et al.*, 2004; Xanthopoulos *et al.*, 2017). This could be attributed to the difference in morphology and surface structures. A great variation in water loss rate is observable among the berry type of fruit due to physiological and structural differences. Climacteric berry types such as feijoas, bananas and tomatoes generally have higher water loss rates compared to the non-climacteric berries such as pomegranates and grapefruit under relatively similar conditions. A quite similar rate of water loss is observed between orange (0.19 % d⁻¹) and pomegranate (0.21 % d⁻¹) fruit under relatively similar low-temperature storage conditions. Pomegranates and oranges are physiologically non-climacteric fruit and botanically classified as berry (hesperidia) fruit with a leathery rind.

Under similar or optimal storage conditions, pomegranate fruit (a berry) tend to have a higher rate of water loss than other fruit such as apples (pome) (**Table 2.1**). During shelf storage, pomegranate lost weight at a rate of 0.19 % d⁻¹ compared to 0.06 % d⁻¹ for apples at 20 °C and 95 % RH, and 0.97 % d⁻¹ compared to 0.22 % d⁻¹ at 20 °C and 65 % RH, respectively (Tu *et al.*, 2000; Mukama *et al.*, 2019). The pomegranates were stored for 30 d while the apples were stored for 18 d. The high susceptibility to water loss of pomegranate fruit is attributed to

the relatively high minute cracks and openings across the skin, despite the relatively thicker rind (Elyatem and Kader, 1984; Nanda *et al.*, 2001; Opara and Al-Ani, 2010). These evenly distributed lenticellular openings are microscopically observable on the epidermis of pomegranate fruit (cv. Hicaznar (Yazici *et al.*, 2011). Likewise, in mangoes moisture loss is related to the density of lenticellular apertures as compared to cuticular waxes in cooking bananas (Ben-Yehoshua and Rodov, 2002). However, micro-cracks and skin openings like lenticels and stomata are not only unique to pomegranate fruit (Berries) but also observed in apples (pomes) (Konarska, 2012). Therefore, moisture loss in fruit is not limited to surface micro-cracks and openings but also occurs across the intact surface layer of the cuticle (Veraverbeke *et al.*, 2003). Besides, micro cracks and openings may clog with wax and suberin materials, becoming unavailable for the free movement of moisture (Veraverbeke *et al.*, 2003). Therefore, to understand water loss differences among fruit types, the effective permeability of the fruit surface layer in addition to determining the density of open functional stomata, micro-cracks and lenticels are important to characterise.

Berry fruit such as tomatoes, eggplants and pepper tend to support more water loss across the calyx than the glossy waxy cuticle that greatly minimises water permeance across the surface skin (Díaz-Pérez, 1998; Nunes and Emond, 2007). About 60 and 67 % of water loss takes place across the calyx in eggplants and tomatoes, respectively, compared to just 2 % in apples (cv. Golden Delicious) (Cameron, 1982; Cameron and Yang, 1982; Díaz-Pérez, 1998). Therefore, special treatments should be considered to minimise moisture loss across the calyx in such fruit, without compromising the integrity and quality of the produce.

Table 2.1 Influence of fruit type on the rate of water loss during storage

Fruit/cultivar	Fruit type	Storage conditions		Water loss (% d ⁻¹)*	Reference
		Temp-time	RH (%)		
High temperature storage (≥ 18 °C)					
Tangerine citrus (cv. Siam Banjar)	Berry (Hesperidia), Non-climacteric	25 °C, 35 d	85-90	1.39	Hassan <i>et al.</i> , 2014
Strawberries (cv. BARI Strawberry-1, Sweet Charlie, Festival, Camarosa & FA 008)	Aggregate fruit, Non-climacteric	25 °C, 4 d	70	3.14-4.27	Rahman <i>et al.</i> , 2016
Pomegranate (cv. Wonderful)	Berry (Hesperidia),	20 °C, 30 d	65 95	0.97 0.19	Mukama <i>et al.</i> , 2019

Fruit/cultivar	Fruit type	Storage conditions		Water loss (% d ⁻¹)*	Reference
		Temp-time	RH (%)		
Apples (cv. Jonagold)	Non-climacteric Pome, climacteric	20 °C, 18 d	65 95	0.22 0.06	Tu <i>et al.</i> , 2000
Plum (cv. Santa Rosa)	Drupe, climacteric	20 °C, 6 d	85	0.85	Guillén <i>et al.</i> , 2013
Peach (cv. Red Heaven)	Drupe, climacteric	20 °C, 6 d	85	1.08	
Pear (cv. Kontoula)	Pome, climacteric	20 °C, 5 d	70 80 95	0.62 0.48 0.24	Xanthopoulos <i>et al.</i> , 2017
Mangoes (cvs. Tommy Atkins & Palmer)	Drupe, climacteric	20 °C, 7-8 d	90-95	0.69-0.74	Nunes <i>et al.</i> , 2007
Cantaloupe melon	Berry (pepos), Non-climacteric	20 °C, 18 d	85-90	0.34	Alhassan and Abdul-rahaman, 2014
Feijoa (cv. Quimba)	Berry, climacteric	18 °C, 7 d	50 70	1.01 0.80	Castellanos <i>et al.</i> , 2016
Medium temperature storage (10 to < 18 °C)					
Pear (cv. Kontoula)	Pome, climacteric	10 °C, 6 d	70 80 95	0.36 0.29 0.09	Xanthopoulos <i>et al.</i> , 2017
Strawberries (cv. Elsanta)	Aggregate fruit, Non-climacteric	15 °C, 5 d	76 86 96	2.40 1.74 1.52	Sousa-Gallagher <i>et al.</i> , 2013
		10 °C, 5 d	76 86 96	2.03 1.49 1.08	
Banana (cv. Dwarf-Prata)	Berry, climacteric	15 °C, 11 d	89	0.10	Maia <i>et al.</i> , 2014
Green Banana (cv. Robusta)	Berry, climacteric	12 °C, 21 d	85-90	0.24	Kudachikar <i>et al.</i> , 2011
Grapefruit (cv. Star Ruby)	Berry (Hesperidia), Non-climacteric	10 °C, 21 d		0.04	Chaudhary <i>et al.</i> , 2012
Tomatoes (cv. Lobello F1)	Berry, climacteric	10 °C, 20 d	70 80 92	0.24 0.11 0.07	Xanthopoulos <i>et al.</i> , 2014
Cantaloupe melon	Berry (pepos), Non-climacteric	9 °C, 18 d	85-90	0.25	Alhassan and Abdul-rahaman, 2014

Fruit/cultivar	Fruit type	Storage conditions		Water loss (% d ⁻¹)*	Reference
		Temp-time	RH (%)		
Low temperature storage (< 10 °C)					
Feijoa (cv. Quimba)	Berry, climacteric	6 °C, 7 d	50	0.47	Castellanos <i>et al.</i> , 2016
			70	0.40	
Pomegranate (cvs. Bhagwa & Ruby)	Berry (Hesperidia), Non-climacteric	5-7 °C, 84 d	90-95	0.12-0.19	Fawole and Opara, 2013
Pomegranate (cv. Wonderful)	Berry (Hesperidia), Non-climacteric	5 °C, 84 d	90-95	0.21	Lufu <i>et al.</i> , 2018
Strawberries (cv. Elsanta)	Aggregate fruit, Non-climacteric	5 °C, 5 d	76	1.65	Sousa-Gallagher <i>et al.</i> , 2013
			86	1.08	
			96	0.82	
Mangoes (cvs. Tommy Atkins & Palmer)	Drupe, climacteric	5 °C, 15-20 d	90-95	0.18	Nunes <i>et al.</i> , 2007
Cucumber	Berry (pepos), Non-climacteric	5 °C	-	0.48	Bahnasawy and Khater, 2014
Oranges (cv. Valencia)	Berry (Hesperidia), Non-climacteric	4 °C, 56 d	80	0.19	Motamedi <i>et al.</i> , 2018
Cantaloupe melon	Berry (pepos), Non-climacteric	2 °C, 18 d	85-90	0.13	Alhassan and Abdul-rahaman, 2014
Plums (cvs. Blackamber & Larry Ann)	Drupe, climacteric	2 °C, 35 d	90	0.17-0.31	Valero <i>et al.</i> , 2013
Apples (cv. Bravo de Esmolfe)	Pome, Non-climacteric	2 °C, 182 d	85	0.04	Rocha <i>et al.</i> , 2004
Peach (cv. Sevilla II)	Drupe, climacteric	2 °C, 28 d	90	0.77	Serrano <i>et al.</i> , 2004
Nectarine (cv. Silver King)	Drupe, climacteric	2 °C, 28 d	90	0.74	
Table grapes (cv. Red Seedless)	Berry, Non-climacteric	1.2 °C, 72 d	89.1	0.10	Ngcobo <i>et al.</i> , 2012
Pear (cv. Kontoula)	Pome, Non-climacteric	0 °C, 8 d	70	0.27	Xanthopoulos <i>et al.</i> , 2017
			80	0.18	
			95	0.06	
Table grapes (cvs. Monukka & Red Globe)	Berry, Non-climacteric	-1 °C, 60 d	90-95	0.06-0.09	Zhang <i>et al.</i> , 2017

*Calculated from text and graphical readings from literature. Temp; temperature, RH: relative humidity, N/A: not available

Cultivar differences affect a wide range of product parameters that directly or indirectly influence water loss during postharvest handling. For the same type of fresh produce, different cultivars have different morphological structures and adaptability to storage stress conditions (**Table 2.2**). These include product size (Monforte *et al.*, 2014), shape (Rodríguez *et al.*, 2011; Monforte *et al.*, 2014) surface pores and micro-cracks (Konarska, 2012), surface roughness (Veraverbeke *et al.*, 2001), cuticular wax content (Chu *et al.*, 2018), respiration rate (Amarante, 1998) and cuticular moisture permeability (Amarante *et al.*, 2001; Vogg *et al.*, 2004). Rahman *et al.* (2016) observed that irrespective of maturity stage and storage duration at 25 °C, water loss was lowest and highest in ‘Camarosa’ and ‘FA 008’ strawberry cultivars, respectively. Similarly, a significantly higher water loss was observed in the ‘Alphonso’ than in the ‘Banganapalli’ mango cultivars (Rao and Shivashankara, 2015). However, in pomegranate fruit, a relatively similar water loss of 20-25 % was observed in cultivars ‘Ruby’ and ‘Bhagwa’ after 28 d at ambient conditions (22 °C and 65 % RH) (Fawole and Opara, 2013). Arendse *et al.* (2014) also observed a water loss of 20 % on ‘Wonderful’ cultivar after 30 d storage at 21 °C and 65 % RH. Furthermore, Al-mughrabi *et al.* (1995) observed no significant difference in water loss for ‘Taeifi’, ‘Banati’ and ‘Manfaloti’ cultivars of pomegranate throughout cold storage at different temperatures, signifying that cultivar may have an insignificant effect on the water loss characteristics of the pomegranate fruit. Similarly, cultivar differences showed no significant influence on the water loss of plums (Valero *et al.*, 2013) and apples (Konopacka *et al.*, 2007). However, it is important noting that most of these studies calculated water loss based on the initial product mass, neglecting the influence of surface area to volume ratio which is an important factor in the rate of water loss among fruit cultivars as discussed below.

Table 2.2 Influence of fruit cultivar on water loss during storage

Fruit	Storage conditions	Cultivars	Water loss (%)	References
Plums	2 °C/90 % RH, 35 d	Blackamber	10.8	Valero <i>et al.</i> , 2013
		Larry Ann	6.1	
Table grapes	-1 °C/90-95 % RH, 60 d	Monukka	5.3	Zhang et al., 2017
		Red Globe	3.6	
Mango	8 °C, 35 d	Alphonso	6.9	Rao and Shivashankara, 2015
		Banganapalli	5.8	
	8 °C, 35 d + 7 d (24-32 °C/60-70 % RH)	Alphonso	15.6	
		Banganapalli	15.1	

Fruit	Storage conditions	Cultivars	Water loss (%)	References
Strawberry	25 °C, 4 d	BARI	50.3	Rahman <i>et al.</i> , 2016
		Strawberry-1		
		Sweet Charlie	67.0	
		Festival	68.3	
		Camarosa	63.7	
Pomegranate	22 °C/65 % RH, 28 d	FA 008	60.0	Fawole and Opara, 2013
		Bhagwa	23.5	
	7 °C/92 % RH, 84 d	Ruby	22.0	
		Bhagwa	17.5	
	5 °C/92 % RH, 84 d	Ruby	16.0	
		Bhagwa	15.9	
Feijoa	4 °C/90 % RH, 21 d	Ruby	11.6	do Amarante <i>et al.</i> , 2017
		Alcântara	2.6	
		Helena	2.9	
		Mattos	1.7	
		Nonante	3.3	
Apples	0 °C/90 % RH, 90 d	Accession 2316	2.4	Konopacka <i>et al.</i> , 2007
		Gala	2.2	
	0 °C/90 % RH, 90 d + 7 d (18 °C/90 % RH)	Jonagold	2.3	
		Gala	3.2	
		Jonagold	3.1	
Pears	-1 to -0.5 °C/92-95 % RH, 180 d	Erica	1.8	Błaszczuk and Łysiak, 2001
		Dicolor	1.4	
	-1 to -0.5 °C/92-95 % RH, 180 d + 7 d (15 °C)	Erica	1.3	
		Dicolor	0.9	
Peach	20 °C, 5 d	October Sun	5.6	Belge <i>et al.</i> , 2014
		Jesca	3.9	

2.2.1.2 Fruit size

Larger fruit are expected to have a lower rate of moisture loss compared to small ones, because of the higher surface area to volume ratio for the later (Ben-Yehoshua and Rodov, 2002). For this reason, smaller apples lose weight faster than larger apples (Maguire *et al.*, 2010) and the neck area of a pear fruit loses weight faster than the bottom side of the fruit (Burger, 2005). Díaz-Pérez (1998) investigated the effect of fruit size on the transpiration rate of eggplants and observed that transpiration rate decreased with increase in product size, partly attributing it to a decrease in the surface area to mass ratio. The authors observed that the effect of product size was significantly more pronounced in smaller eggplants (< 0.10 kg per fruit) than in larger ones (> 0.10 kg per fruit). Interestingly, a double moisture loss rate is reported in size-32 (1.12 % kPa⁻¹ d⁻¹) compared to size-16 (0.62 % kPa⁻¹ d⁻¹) of the eggplants. Therefore, it is more practical

to report the fresh produce transpiration rate per unit surface area rather than per fruit mass (Burton, 1982; Bovi *et al.*, 2016) as applied in transpiration studies of mushrooms (Mahajan *et al.*, 2008), grape tomatoes (Xanthopoulos *et al.*, 2014), pears (Xanthopoulos *et al.*, 2017) and recently pomegranates (Lufu *et al.*, 2019).

2.2.2 Orchard practices

Table 2.3 summarises the influence of orchard management practices on water loss of fruit during different storage conditions. Several orchard cultural practices applied intending to increase yield and enhance other produce qualities might as well directly or indirectly influence produce water loss during postharvest storage.

As summarised in **Table 2.3**, Zhang *et al.* (2017) observed significantly minimised water loss in two cultivars of table grapes grown with silicate fertilizer application compared to control fruit grown without fertilizer application. Similarly, the application of phosphorus and potassium effectively minimised water loss in ‘Ponkan’ citrus fruit compared to control fruit grown without fertilizer application (Xian-yong *et al.*, 2006). This can be attributed to the ability of phosphorus to minimise respiration rate of fresh produce. However, the use of nitrogen fertilizers showed no significant influence on water loss between broccoli (cv. Italica) grown with and without fertilizer application during storage at 1 °C and > 95 % RH for 20 d (Toivonen *et al.*, 1994).

Application of foliar spray before harvesting minimises moisture loss as observed in grapefruit (cv. Ruby red) during storage (Fucik, 1982). This has been attributed to the preservation of fruit firmness and tissue integrity by minimising enzymatic disintegrations of cellular structure. Furthermore, using sprays containing calcium, magnesium and titanium ions significantly minimised water loss in peaches and nectarines (Serrano *et al.*, 2004; Gayed *et al.*, 2017) as summarised in **Table 2.3**. Treatment with calcium and magnesium preserves the functionality of tissues by minimising ion leakage, protein and phospholipid loss (Lester and Grusak, 1999). Although pre-harvest application of antiperspirants helps in providing a barrier against water loss and or facilitating closure of stomata, this is at the expense of gaseous exchange and photosynthesis (Ben-Yehoshua and Rodov, 2002). Therefore, careful application is necessary to avoid anaerobic respiration.

A sudden change in the water status of the fruit trees can initiate fruit cracking, enhancing fruit moisture and water loss in apples (Maguire *et al.*, 2010). Fruit cracking is most likely to occur if the fruit is harvested immediately after an irrigation (Volz *et al.*, 1993).

Immediate harvesting of fresh produce shortly after an irrigation can result in faster rates of water loss due to high turgidity of cells and higher vapour pressure deficit, especially before cold storage. Fully irrigated blueberries (cv. Brigitta) lost 10 to 15 % more weight than severely deficit irrigated fruit, after 30 d of storage at 0 – 2 °C, for fruit grown in Colbún during the 2013/14 and 2014/15 seasons (Lobos *et al.*, 2016). Similar results were observed in pomegranates (Laribi *et al.*, 2013; Peña *et al.*, 2013) and grapefruit (Romero-Trigueros *et al.*, 2017) as summarised in **Table 2.3**. Overly turgid cells may facilitate splitting and rupture in vegetables and fruit increasing surface area across which, moisture is lost. Application of deficit (below optimum) irrigation results into a more continuous and thicker cuticular layer of the fruit compared to the more cracked and thinner layer in overly irrigated fruit (Crisosto *et al.*, 1994; Peña *et al.*, 2013). Furthermore, restricted irrigation promotes increased growth and density of trichomes on the produce surface, which intern supports reduced water loss during postharvest conditions (Crisosto *et al.*, 1995).

Table 2.3 The impact of orchard management practices on fruit water loss during storage

Factor	Fruit	Storage conditions	Application	Water loss (%)	Remarks	References
Irrigation	Pomegranate (cv. Mollar de Elche)	5 °C/90 % RH, 90 d	Fully irrigated (100 % evapotranspiration)	14.7	Deficit irrigation significantly minimised fruit water loss during storage compared to fully irrigated fruit.	Peña <i>et al.</i> , 2013
			Sustained deficit irrigation (32 % evapotranspiration)	10.6		
	Pomegranate (cv. Mollar de Elche)	5 °C/90-95 % RH, 133 d + 7 d (20 °C/70-75 % RH)	Fully irrigated (100 % evapotranspiration)	21.0	Restricted irrigation significantly minimised water loss during storage compared to fully irrigated fruit	Laribi <i>et al.</i> , 2013
			Sustained deficit irrigation (50 % evapotranspiration)	17.6		
			Severe water restriction at flowering (25 % evapotranspiration)	19.8		
			Severe water restriction at growth (25 % evapotranspiration)	19.8		
	Grapefruit (cv. Star Ruby)	10 °C/85 % RH, 31 d	Severe water restriction at ripening (25 % evapotranspiration)	17.6	Deficit irrigation significantly minimised fruit water loss during storage compared to fully irrigated fruit	Romero-Trigueros <i>et al.</i> , 2017
			Fully irrigated (100 % evapotranspiration)	8.0-8.5		
			Regulated deficit (50 % evapotranspiration)	7.0-7.4		
	Blueberry (cv. Brigitta) of Colbún 2013-14 season	0-2 °C, 60 d + 3 d (18-0 °C)	Severe water deficit (50 % evapotranspiration, ETa)	15.6	water loss was significantly higher in fully irrigated fruit than in water-stressed fruit	Lobos <i>et al.</i> , 2016
			Mild water deficit (75 % evapotranspiration, ETa)	15.3		
			No-water deficit (100 % evapotranspiration)	16.4		

Factor	Fruit	Storage conditions	Application	Water loss (%)	Remarks	References
Foliar spray	Peach (cv. Early Swelling), 2015 season	0 °C/85-90 % RH, 35 d	Control (untreated)	33.7	Spraying with calcium chloride and or chitosan significantly minimised water loss, compared to untreated fruit	Gayed <i>et al.</i> , 2017
			Sprayed (Calcium chloride, 1 & 2 %)	24.3		
			Sprayed (Chitosan, 0.5 & 1 %)	22.7		
			Sprayed (Calcium chloride & Chitosan)	19.7		
	Peach (cv. Sevilla II)	2 °C/90 % RH, 28 d	Control (untreated)	21.7	A lower water loss in the treated fruit compared to untreated fruit	Serrano <i>et al.</i> , 2004
			Sprayed (spray containing Ca ²⁺ , Mg ²⁺ & Ti ⁴⁺)	15.8		
Fertilizer application	Nectarine (cv. Silver King)	2 °C/90 % RH, 28 d	Control (untreated)	20.8	A lower water loss in the treated fruit compared to untreated fruit	Serrano <i>et al.</i> , 2004
			Sprayed (spray containing Ca ²⁺ , Mg ²⁺ & Ti ⁴⁺)	13.6		
	Table grapes (cv. Monukka)	-1 °C/90-95 % RH, 60 d	No-fertilizer (control)	5.3	Using silicate fertilizers significantly minimised water loss in treated fruit compared to untreated control fruit.	Zhang <i>et al.</i> , 2017
			Fertilizer (Steel slag, 25 % silicate)	4.0		
			Fertilizer (Water-cooling slag, 18 % silicate)	3.8		
	Table grapes (cv. Red Globe)	-1 °C/90-95 % RH, 60 d	No-fertilizer (control)	3.6	Using silicate fertilizers significantly minimised water loss in treated fruit compared to untreated control fruit.	Zhang <i>et al.</i> , 2017
			Fertilizer (Steel slag, 25 % silicate)	3.0		
			Fertilizer (Water-cooling slag, 18 % silicate)	2.8		

2.3 Harvest factors

2.3.1 Fruit maturity

Matured produce have well-developed peel (outer) structures and these act as barriers to moisture loss (Lendzian and Kerstiens, 1991). Therefore, immature fruit and vegetables lose more moisture than commercially mature products (Sastry, 1985). This is supported by finding from Chomchalow *et al.* (2002) who observed minimum water loss in non-ethylene treated most matured tomatoes (cv. Sunny) stored at 12.5 °C for 1 d compared to medium and least matured tomatoes. Also, during forced-air curing of sweet onions at 35 to 38 °C, 20 to 30 % RH and air velocity of 0.3-1.0 m s⁻¹ for 1-4 d, early harvested bulbs lost 10.1 % compared to 7.3 and 5.9 % for optimal and late maturity harvested bulbs, respectively (Maw and Mullinix, 2005).

On the contrary, Rahman *et al.* (2016) observed higher water loss (14.6 %) in five cultivars of fully matured strawberries after 4 d of storage at 25 °C, than in two thirds (11.8 %) and one-third (7.0 %) matured fruit, respectively. Also, an increase in water loss with increasing level of maturity was observed in persimmon (cv. Natanzy) fruit throughout 140 d of storage at 2 °C, with first harvest (early maturity) and fourth harvest fruit losing less than 10 % and well above 16 %, respectively (Ramin and Tabatabaie, 2003). Similarly, water loss of sweet bell peppers (cvs. Velez and Telmo) progressively increased with increasing maturity level during refrigeration storage for 28 d (Tsegay *et al.*, 2013). Furthermore, overly mature fruit also lose more moisture than commercially mature fruit (Sastry, 1985; Theron, 2015). This is attributed to changes in the structure of the outer layers, skin coatings and cracking of cuticle layers (Burton, 1982; Jenks and Ashworth, 2010). A two-fold increase was reported in cuticle permeability of different cultivars of plums, as the fruit became overly mature (Theron, 2015). Therefore, it is important to harvest fruit and vegetables at their optimal maturity to minimise postharvest water losses during storage.

2.3.2 Weather condition and climatic season

The harvest seasons of different fresh produce worldwide coincides with different weather conditions such as sunshine intensity, wind speed, rainfall and temperature, and climatic seasons such as dry, hot, wet and cold seasons. The bulk of heat that comes with fruit harvested on a hot time of the day can trigger high respiration and transpiration rates, resulting into rapid water loss and quality deterioration during prolonged storage (Ravindra and Goswami, 2008;

Tyagi *et al.*, 2017). Therefore, it is recommended that a pre-cooling treatment is applied immediately after harvest prior to prolonged storage (Brosnan and Sun, 2001; Ravindra and Goswami, 2008). Furthermore, in case of hot days and seasons, harvesting of fresh produce is commonly carried out very early in the morning or late in the evening when the environment is naturally cooler than the mid-day.

On the other hand, fruit harvested immediately after heavy rainfall is more susceptible to high rates of water loss during storage, due to increased turgor pressure within the product tissues as discussed in the previous **Section (2.2)**. In the case of such scenarios, subjecting fruit to conditions that allow the loss of excess moisture is vital. For example, conditioned moisture loss of about 3 % prevents fruit decay and minimises further moisture loss in citrus fruit (Murata and Yamawaki, 1992).

2.3.3 Harvest technique and physical injury

There has been a shift in the application of horticultural practices, from simple labour intensive to mechanised systems to support planting and harvesting (Li *et al.*, 2011) owing to the elevated demand for fruit and vegetables worldwide (Li and Thomas, 2014). The increased mechanisation increases the susceptibility of injuring fresh produce during their development and harvesting periods (Kays, 1999; Lurie, 2009). Mechanised harvesting that applies various forces to the fruit such as impact, vibration and compression forces may cause fruit damage and therefore, needs to be controlled (Li and Thomas, 2014). Overfilling of bins and bags may compress the fruit (Thompson, 2003). Damaging of fresh produce peel cover before or during harvesting causes increase in the peel permeance, moisture flux and loss during storage, due to disorientation of surface tissues (Wills, 1998).

Elshiekh and Abu-Goukh (2008) investigated two harvesting methods on grapefruit (cv. Foster). The fruit harvested using the traditional method (Hook on a pole) had a significantly higher water loss (51 % more) compared to fruit harvested with the improved method (ringed long cloth sleeve on a pole). This was attributed to the bruising of fruit due to the falling impact on the ground. These results are strengthened by findings from Thomson (2005) in studying different harvest techniques and injury level on water loss of pak choi (*Brassica rapa* subsp. *Chinensis*, cv. Sumo), stored at 15 °C. Significantly higher water loss was observed on products harvested by method 1 (traditionally harvested by a slight cut through the bases of the petioles) compared to an improved method 2 (cutting through the taproot). This was attributed to the harvest wounds induced by method 1, which contributed 20

% to the total water loss of the product. Furthermore, water loss was observed to increase with increasing level of induced injury. However, in attempting to minimise harvest wounds, a significantly higher water loss was observed in products with intact root systems, attributing it to increased surface area for accelerated moisture loss. In such a scenario, a compromise that creates minimal injury to the product is needed to minimise excessive water loss during storage.

2.3.4 Tree age and canopy position

The effect of tree age on postharvest water loss of fruit was demonstrated on mangoes (cv. Amrapali (Meena and Asrey, 2018)). Physiological water loss of fruit harvested from 30-year-old trees (17.7 %) was significantly higher than in fruit from 6-year-old (14.8 %) and 18-year-old (15.9 %) trees, at the end of 9 d storage at 35 ± 3 °C and 60 ± 5 % RH. The differences in water loss were attributed to lower respiration rate, enzymatic activity (pectin methylesterase and polygalacturonase), ethylene production rate, lenticel density and higher peel thickness in fruit harvested from younger trees than from older trees. Tree age and canopy position were reported to have a significant effect on the rind quality of mandarin fruit (cv. Kinnow), with peel thickness being lowest and highest in fruit harvested from 35-year-old and 2-year-old trees, respectively (Khalid *et al.*, 2012).

Fruit harvested from the top and more exposed canopy positions are most likely to lose less water during postharvest storage compared to fruit harvest from lower and less exposed canopy positions. Probably because fruit size and dry matter proportion tend to decrease from the top to the lower region of the canopy, as observed in mangoes (cv. Kensington (Hofman *et al.*, 1995)). Fruit size and dry matter influence postharvest water loss of stored fruit as discussed in previous **Section 2.1.2**. In addition, total sugar and total soluble content which contribute to fruit dry matter content were observed to be more in top canopy positioned fruit than in lower positioned ones (Mendoza *et al.*, 1984). Furthermore, fruit and vegetables exposed to climate conditions of light, sunshine and wind tend to have low stomata density as compared to less exposed ones.

2.4 Postharvest factors

2.4.1 Temperature

Temperature is one of the most important factors affecting moisture loss from fresh produce, having an impact on water potential of the produce and relative humidity of the surrounding,

increasing the water potential deficit between the product and its environment (Gahan, 1999). Secondly, a high product temperature provides more energy to facilitate evaporation of water from the surfaces while as an increase in atmospheric temperature increases the water holding capacity of the surrounding air and this facilitates the moisture loss (Ben-Yehoshua and Rodov, 2002). Thirdly, the permeability of the peel (skin) layers of fruit and vegetables increases with increasing temperature (Nguyen *et al.*, 2006). This is attributed to the re-orientation of the lipids of the cuticles and creation of hydrophilic openings in the barrier layers (Eckl and Gruler, 1980). Lastly, high temperatures facilitate increased depletion rate of respiration substrates and moisture diffusion across product peel (Caleb *et al.*, 2012b; Xanthopoulos *et al.*, 2017; Bovi *et al.*, 2018).

The influence of storage temperature on water loss is demonstrated in the following studies on pomegranate fruit. A water loss of 27.7 and 45.7 % were observed in pomegranate (cv. Wonderful) stored at 5 °C and 7.5 °C respectively, for 150 d at 92 % RH (Arendse *et al.*, 2014). Elyatem and Kader (1984) reported 1.0, 1.4, 1.6, and 2.7 % water loss for pomegranate fruit (cv. Wonderful) after 35 d of storage at 0, 5, 10 and 20 °C, respectively, and 96 % RH. Likewise, Fawole and Opara (2013) observed significantly higher water loss for pomegranate (cv. Ruby and Bhagwa) stored at 10 °C and 92 % RH compared to fruit stored at 5 °C/92 % RH and 7 °C/92 % RH throughout 84 d of storage, attributing it to differences in respiration rate. Similar results are reported in other fruit and vegetables like pears (Xanthopoulos *et al.*, 2017), strawberries (Bovi *et al.*, 2018), mushrooms (Mahajan *et al.*, 2016), grape tomatoes (Xanthopoulos *et al.*, 2014) and table grapes (Pereira *et al.*, 2017). It is reported that small changes in storage temperature cause fluctuations in RH, supporting moisture loss of produce during refrigeration (Koca *et al.*, 1994). The combination of high temperature and low humidity causes high respiration and transpiration in fruit (Opara *et al.*, 2008), leading to higher water loss. It is therefore important that fruit is precooled immediately after harvest and subsequently in maintaining the optimum temperature and humidity throughout the cold chain: storage, transport and marketing (Thompson *et al.*, 2008).

2.4.2 Relative humidity

At a constant temperature, small changes in relative humidity can significantly affect the rate of water loss in fresh produce (Ben-Yehoshua and Rodov, 2002). A lower RH increases the water vapour pressure deficit (WVPD) across the fruit peel, increasing the moisture diffusion

from the fruit to the surrounding air (Paull, 1999; Ngcobo *et al.*, 2013). Theron (2015) also observed that high WVPD resulted in high moisture loss for different cultivars of plums stored at ambient temperatures for 3 d. The ranges of water losses of different fruit as a function of humidity are summarised in **Table 2.4**. A significantly higher water loss of 29.1 % was observed in pomegranate fruit (cv. Wonderful) stored at 65 % RH compared to 5.8 % loss for fruit stored at 95 % RH after 30 d of storage at 20 °C (Mukama *et al.*, 2019). These results are further strengthened by findings from other studies on pomegranate (Lufu *et al.*, 2019), pears (Xanthopoulos *et al.*, 2017), strawberries (Sousa-Gallagher *et al.*, 2013; Bovi *et al.*, 2018), pomegranate arils (Caleb *et al.*, 2013b), mushrooms (Mahajan *et al.*, 2016; Sousa-Gallagher *et al.*, 2013), grape tomatoes (Xanthopoulos *et al.*, 2014) and table grapes (Pereira *et al.*, 2017).

To minimise water loss through transpiration, the water content of the environment should be kept as close as possible to the water content of the product. It is therefore clear that postharvest water loss is greatly minimised by manipulating the relative humidity during cold and shelf storage. With most fresh produce having 85 to 95 % water content, an atmosphere of 90 to 95 % RH is recommended (Ben-Yehoshua and Rodov, 2002). Elyatem and Kader (1984) suggested an RH of 95 % and above to minimise water loss in pomegranate fruit (cv. Wonderful) which are susceptible to moisture loss across numerous pores on the fruit peel. The Authors observed that decreasing relative humidity from 95 to 75 % at 5 °C will increase fruit water loss from 1.4 to 6.1 %. However, it is important to note that moisture loss continues to take place even at 100 % RH condition in the form of perspiration through the openings on the peel and sweat (condensates) on the fruit surface (Gahan, 1999; Bovi *et al.*, 2018). Hence, very high relative humidity can cause moisture condensation on fruit surfaces. This creates favourable conditions for the proliferation of microbial growth leading to fruit decay (Ben-Yehoshua *et al.*, 1988; Lufu *et al.*, 2018).

Table 2.4 Influence of storage relative humidity (RH) on fruit water loss

Fruit	Storage condition	RH (%)	Water loss (%)*	References
Pomegranate (cv. Wonderful)	20 °C, 30 d	65	29.1	Mukama <i>et al.</i> , 2019
		95	5.8	
Pomegranate (cv. Wonderful)	25 °C, 43 d	77	6.6	Lufu <i>et al.</i> , 2019
		82	16.6	
		93	18.0	
Apples (cv. Jonagold)	20 °C, 18 d	30	6.0	Tu <i>et al.</i> , 2000
		65	4.0	

Apples (cv. Braeburn)	20 °C, 18 d	95	1.0	Tu <i>et al.</i> , 2000
		30	5.3	
		65	3.8	
Grape tomatoes (cv. Lobello F1)	10 °C, 20 d	95	1.0	Xanthopoulos <i>et al.</i> , 2014
		70	4.8	
		80	2.2	
		92	1.4	
Pear (cv. Kontoula)	20 °C, 5 d	70	3.1	Xanthopoulos <i>et al.</i> , 2017
		80	2.4	
		95	1.2	
	10 °C, 6 d	70	2.2	
		80	1.8	
		95	0.5	
	0 °C, 8 d	70	2.2	
		80	1.3	
		95	0.5	
Feijoa (cv. Quimba)	18 °C, 7 d	50	7.1	Castellanos <i>et al.</i> , 2016
		70	5.6	
	6 °C, 7 d	50	3.3	
		70	2.8	
Strawberries (cv. Elsanta)	5 °C, 5 d	76	3.4	Sousa-Gallagher <i>et al.</i> , 201
		86	2.3	
		96	1.7	
	10 °C, 5 d	76	4.2	
		86	3.1	
		96	2.3	
	15 °C, 5 d	76	5.0	
		86	3.6	
		96	3.2	

*Calculated from text and graphical readings from literature. RH: relative humidity

2.4.3 Storage duration

Fruit and vegetables continue to undergo respiration and transpiration, utilising sugar and water reserves yet without replacement and therefore continue to lose turgidity and weight, resulting into shrivelling and shrinkage. Usually, a higher rate of water loss is observed at the beginning of the water loss profile, which then decreases with storage duration (Burdon and Clark, 2001). The beginning stage of the water loss profile is the most importantly studied in mimicking storage and marketing conditions of the fruit as compared to the later stage. In this case, many studies have reported a progressive increase in water loss with storage duration for various fruit and vegetable (Caleb *et al.*, 2013a; Xanthopoulos *et al.*, 2014, 2017; Castellanos *et al.*, 2016; Lufu *et al.*, 2018, 2019). **Table 2.1** shows the rate of water loss in various fruit types at different

storage conditions. Furthermore, a significant ($P < 0.0001$) interaction impact of storage duration and temperature is reported in pomegranate fruit cultivars (Fawole and Opara, 2013; Lufu *et al.*, 2019).

The decreasing rate of water loss in the later stage of the water loss profile can be attributed to senescence and hardening of the outer layer of the peel, reducing its permeability to water. However, a reduction in cuticular wax content and composition observed in different fruit cultivars of blueberries with time (Chu *et al.*, 2018), may imply an increase in moisture permeability across peels. Therefore, over the past decades, researchers have been investigating various technologies such as modified atmosphere packaging, surface coating, chemical and heat treatments to minimise water loss during prolonged cold storage.

2.4.4 Airflow

Prior to storage, it is important to remove the field and respiratory heat from fresh produce as efficiently as possible using a pre-cooling process such as forced air-cooling (Thompson *et al.*, 2008). A higher flow rate of the cooling air means faster cooling of the produce. However, a higher flow rate causes a high rate of water loss, signifying that optimizing the airflow velocity is crucial. Mukama *et al.* (2019) reported water loss of 0.01 to 0.06 % h^{-1} during forced-air cooling of pomegranate fruit (cv. Wonderful) packed with and without plastic liners. Generally, the moisture transfer coefficient increases with the increase in velocity of air when it flows over the produce (reducing the boundary layer thickness, increasing diffusion across peel) (Gaffney *et al.*, 1985; Maguire *et al.*, 2010). The influence of airflow velocity on water loss is more prominent at lower vapour pressure deficit between fruit and the surrounding atmosphere. Whitelock *et al.* (1994) observed that the influence of both high and negligible airflow was generally greater at lower vapour pressure deficit for the different cultivars of peach fruit. A predictive model demonstrated the effect of air velocity on moisture loss during a drying process under different RH (40-10 %) conditions where moisture loss increased rapidly with increasing air velocity (0-5 m s^{-1}) followed by a slowing down until becoming constant (Bahnasawy and Shenana, 2004). For the above reasons, the use of optimised ventilated cartons and perforated plastic liners is encouraged to balance between faster cooling and minimised water loss (Mukama *et al.*, 2019).

2.4.5 Packaging

Tables 2.5 and **2.6** summarise the role of plastic liners and heat shrinkable films in water loss of produce during postharvest storage conditions, respectively. Most importantly, the barrier properties of most packaging material facilitate moisture build-up around the fruit, increasing relative humidity and reducing vapour pressure deficit across the peel (Suparlan and Itoh, 2003; Ngcobo *et al.*, 2013). Likewise, packaging provides a barrier against airflow and minimises the rate at which moisture is carried away from around the fruit, minimising diffusion across peel, especially for non-perforated liners, as compared to perforated liners (Gaffney *et al.*, 1985; Maguire *et al.*, 2010). Furthermore, the ability of modified atmosphere packages (MAP) to alter gaseous atmosphere around the fruit and vegetables, reduces water loss by minimising respiration rate (Selcuk and Erkan, 2014; Lufu *et al.*, 2018).

After 35 d of storing ‘Regal seedless’ table grapes at -0.5 °C and 95 % RH, fruit packed in bunch carry-bag packaging combination lost 1.1 % of initial weight compared to 2.0-3.1 % for fruit packed in open-top punnets and clamshell combinations (Ngcobo *et al.*, 2013). The authors attributed the results to the difference in relative humidity maintained around the fruit by the packaging. In another study, packing pear (Punjab Beauty) in non-perforated HDPE liners inside corrugated cartons significantly minimised weight to about 3.5 % after 75 d of cold storage at 0-1 °C and 90-95 % RH compared to 6 % for fruit packed in cartons alone (Kaur *et al.*, 2013). It is important to note that the application of plastic liners in cold chain management of fruit water loss has come with challenges such as moisture condensation and associated fruit decay (Ben-Yehoshua *et al.*, 1988; Lufu *et al.*, 2018). Micro and macro perforated liners have been applied to counteract these arising challenges and **Table 2.5** shows the impact of perforated liners on fruit water loss as compared to non-perforated liners. Perforation minimises vapour condensation in MAP liners, due to changes in vapour transmission capability of the liners (Ben-Yehoshua *et al.*, 1988; Opara *et al.*, 2015; Lufu *et al.*, 2018). However, the movement to reduce and stop the use and reuse of recyclable plastics is a growing problem in the fruit and vegetable industry currently, thus suggesting the use of other moisture loss controls such as surface coating.

Table 2.5 Effect of plastic liner packaging on water loss of stored fruit

Product	Storage conditions	Plastic liner application	Water loss (%)	Reference
Grape bunches (cv. Red Seedless)	1.2 °C/89.1 % RH, 72 d	No-liner	7.3	Ngcobo <i>et al.</i> , 2012
		Non-perforated liner	0.4	
		Perforated liner	5.2-6.4	
Plums (cv. Friar)	0 °C/85 % RH, 60 d	No-liner	6.0	Canti'n <i>et al.</i> , 2008
		Non-perforated liner	0.1-0.3	
		Perforated liner	2.0	
Pomegranates (cv. Hicrannar)	6 °C/90 % RH, 120 d	No-liner	17.2	Selcuk and Erkan, 2014
		Non-perforated liner	1.5-4.4	
Feijoa (cv. Quimba)	6 °C/50 % RH, 7 d	No-liner	3.3	Castellanos <i>et al.</i> , 2016
		Liner (1 perforation)	0.5	
		Liner (2 perforations)	0.5	
Grapefruit (cv. Star Ruby)	10 °C/80-85, 112 d + 7 d (20 °C)	No-liner	4.2	Chaudhary <i>et al.</i> , 2015
		Non-perforated liner	3.3	
		Perforated liner	3.7	
Sweet cherry (cvs. Bing & Sweetheart)	0 °C, 42 d	Non-perforated liner	<1.0	Wang and Long, 2014
		Perforated liner	<1.0	
Kiwifruit (cv. California)	0 °C, 120 d	Non-perforated liner	0.8	Wiley <i>et al.</i> , 1999
		Perforated liner	3.0-4.5	

Table 2.6 Influence of shrink wrapping on fruit water loss during storage

Product	Storage conditions	Surface wrapping application	Water loss (%)	Reference
Apples (cv. Royal delicious)	22-28 °C/52-68 % RH, 42 d	No-film control	18.0	Sharma <i>et al.</i> , 2013
		Heat Shrinkable film (Cryovac, 9 µ)	4.3	
		Heat Shrinkable film (Polyolefin, 13 µ)	5.3	
		Heat Shrinkable film (LDPE, 25 µ)	7.7	
Pomegranate (cv. Wonderful)	7 °C/92 % RH, 90 d	No-film control	16.3	Mphahlele <i>et al.</i> , 2016
		Heat shrinkable film (double-layered co-extruded polyolefin, 0.025 µm) polyliner	2.0	
			2.0	
Pomegranate (cv. Primosole)	8 °C/90 % RH, 42 d	No-film control	5.1	D'Aquino <i>et al.</i> , 2010
		Heat Shrinkable film (polyolephenic, 24 µm)	0.6	
		No-film control	~22.0	Rao, 2018

Pomegranate (cvs. Mridula & Bhagwa)	25-32 °C/49-67 % RH	Heat shrinkable film (D-955, multilayered	< 2.0	Rao and Shivashankara, 2015
		Cross-linked polyolefin, 25 µm)		
		Heat shrinkable film (BDF-2001, multilayered	< 2.0	
		Co-extruded polyolefin, 25 µm)		
Mango (cv. Alphonso)	8 °C, 35 d + 7 d (24- 32 °C/60- 70 % RH)	Heat shrinkable film (LDPE, 25 µm)	< 2.0	Rao and Shivashankara, 2015
		No-film control	15.6	
		Heat shrinkable film (D-955, multilayered	9.0	
		Cross-linked polyolefin, 15 µm)		
Mango (cv. Banganapalli)	8 °C, 35 d + 7 d (24- 32 °C/60- 70 % RH)	Heat shrinkable film (LD-935, multilayered	9.1	Rao and Shivashankara, 2015
		Cross linked polyolefin, 11 µm)		
		No-film control	15.1	
		Heat shrinkable film (D-955, multilayered	8.3	
		Cross-linked polyolefin, 15 µm)		
		Heat shrinkable film (LD-935, multilayered	7.9	
		Cross-linked polyolefin, 11 µm)		
		Heat shrinkable film (LDPE, 25 µm)	8.0	

2.4.6 Surface coating

The role of surface waxing and coating in the water loss of stored fruit is summarised in **Table 2.7**. In addition to minimising water loss, waxing noticeably improves the visual appearance of the fruit as demonstrated in **Figure 2.2** (Motamedi *et al.*, 2018). Generally, fruit and vegetable outer layers naturally have waxes on their cuticle that help minimise moisture loss. Surface coating and waxing are applied to replace the natural waxes that are lost during the washing and cleaning of products (Bahnasawy and Khater, 2014). Surface coatings help to cover micro-cracks on the skin, acting as a barrier to surface permeance (Banks *et al.*, 1997). The effectiveness of the coating and waxing depends on the concentration (Amarante *et al.*, 2001). Bahnasawy and Khater (2014) observed decreasing water loss (0.020 to 0.005 % h⁻¹) with increasing concentration of paraffin wax solution (0 to 100 %) for cucumber stored at different temperatures. Waxing of bunch tomatoes (cv. Bandita) with tomato wax (99 % food-grade mineral oil and 1 % water) tremendously minimised water loss compared to using nutrient solution, herbal oil and un-waxed treatments after 16 d of storage at 20 °C and 90 % relative humidity (Dilmaçunal *et al.*, 2011). Over years, edible surface coating and waxing have been successfully applied on apples, pomegranates, mangoes, plums, blueberries and oranges to minimise moisture loss (Nisperos-Carriedo *et al.*, 1990; Park *et al.*, 1994; D'Aquino *et al.*, 2012; Soomro *et al.*, 2013; Valero *et al.*, 2013; Vieira *et al.*, 2016; Motamedi *et al.*, 2018). However, it is important to note that very thick coating may result in anaerobic respiration producing undesirable ethanol associated off flavours, suggesting that consideration should be taken for product physiology (Ben-Yehoshua and Rodov, 2002; Motamedi *et al.*, 2018).

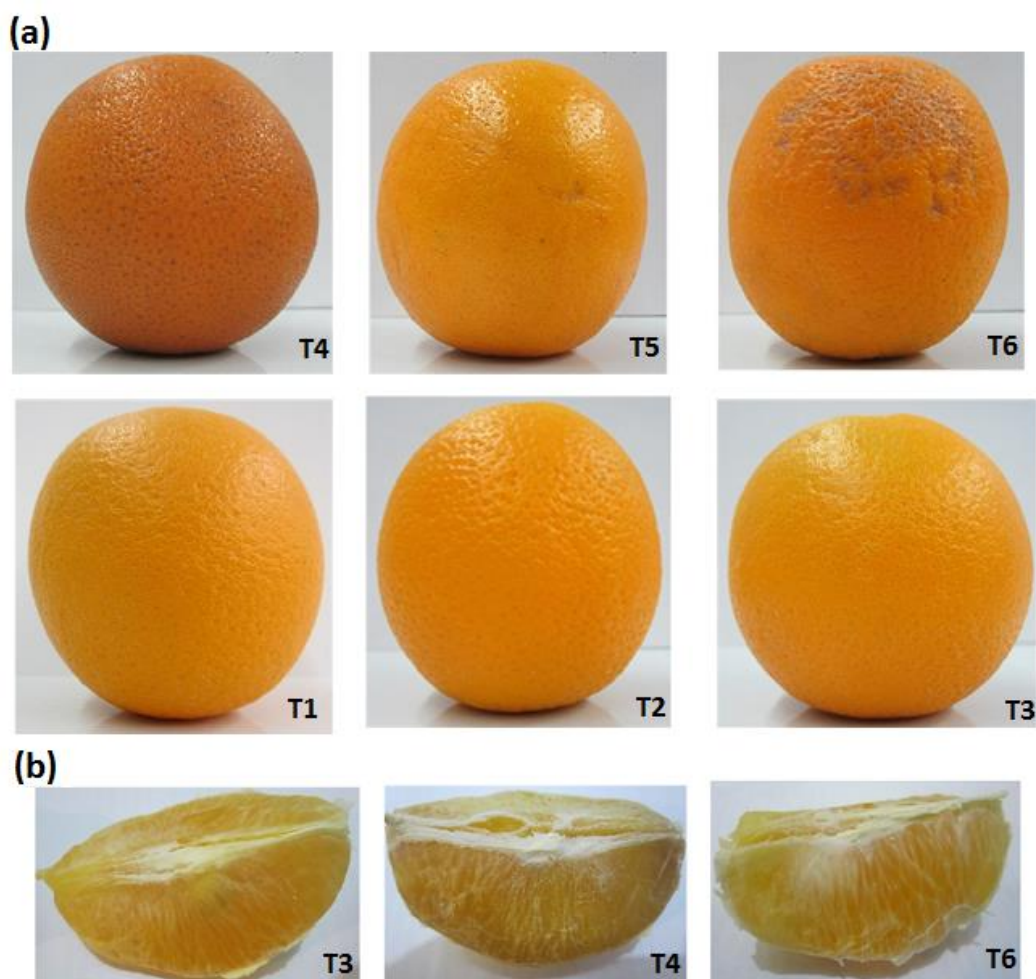


Figure 2.2 Effects of wax coating on the outer (a) and inner (b) visual appearance of ‘Valencia’ orange fruit after 56 d of cold storage at 4 °C with additional 7 d storage at 20 °C (Motamedi *et al.*, 2018). Synthesised carnauba wax with 0.0 % (T1), 0.5 (T2) and 1.0 % (T3) organoclay, commercial wax 1 (T4) and wax 2 (T5) and no wax control fruit (T6).

Table 2.7 Effect of postharvest surface coating in controlling fruit water loss

Product	Storage conditions	Surface coating/waxing application	Water loss (%)	Reference
Mango (cv. Langra)	25 °C, 30 d	No-waxing	6.0	Soomro <i>et al.</i> , 2013
		Coated (sunflower wax)	4.1	
	4 °C	No-waxing	5.0	
		Coated (sunflower wax)	2.5	
Tangerine citrus (cv. Siam Banjar)	25 °C, 35 d	No-waxing	48.5	Hassan <i>et al.</i> , 2014
		Coated (bees wax emulsion, 10 %)	38.0	
		Coated (bees wax emulsion, 12 %)	31.0	
		Coated (bees wax emulsion, 15 %)	30.1	
		No-waxing	10.8	

Plums (cv. Blackamber)	2 °C/90 % RH, 35 d	Coated (1 % alginate-based coating)	8.4	Valero <i>et al.</i> , 2013
		Coated (3 % alginate-based coating)	5.8	
Plums (cv. Larry Ann)	2 °C/90 % RH, 35 d	No-waxing	6.1	Guillén <i>et al.</i> , 2013
		Coated (1 % alginate-based coating)	5.2	
		Coated (3 % alginate-based coating)	5.0	
Plum (cv. Santa Rosa)	20 °C/85 % RH, 6 d	No-waxing	5.1	
		Coated (Aloe vera gel)	2.5	
		Coated (Aloe arborescens gel)	1.4	
Peach (cv. Red Heaven)	20 °C/85 % RH, 6 d	No-waxing	6.5	Guillén <i>et al.</i> , 2013
		Coated (Aloe vera gel)	5.2	
		Coated (Aloe arborescens gel)	3.8	
Pomegranate (cv. Primosole)	8 °C/90-95 % RH, 84 d	No-waxing	8.4	D'Aquino <i>et al.</i> , 2012
		Coated (Soy lecithin)	6.8	
	8 °C/90-95 % RH, 84 d + 7 d (20 °C/65-70 % RH)	No-waxing	12.8	Motamedi <i>et al.</i> , 2018
		Coated (Soy lecithin)	11.0	
Oranges (cv. Valencia)	4 °C/80 % RH, 56 d	No-waxing	10.4	
		Commercial wax 2	4.8	
		Commercial wax 1	5.8	
		Synthesised carnauba 1.0 % organoclay	4.0	
		Synthesised carnauba 0.5 % organoclay	4.3	
		Synthesised carnauba 0.0 % organoclay	4.7	
	4 °C/80 % RH, 56 d + 7 d (20 °C)	No-waxing	14.9	
		Commercial wax 2	9.1	
		Commercial wax 1	8.9	
		Synthesised carnauba 1.0 % organoclay	6.0	
		Synthesised carnauba 0.5 % organoclay	6.8	
		Synthesised carnauba 0.0 % organoclay	7.6	
Blueberry (cv. Duke)	5 °C, 25 d	No-waxing	6.2	Vieira <i>et al.</i> , 2016
		Coated (Chitosan coating)	5.1	
		Coated (Chitosan-Aloe vera coating)	3.7	

2.4.7 Other pre-treatments

Several other postharvest factors are influencing produce water loss, summarised in **Table 2.8**. The application of calcium through fertilizers and foliar sprays has been reported to increase yield in various crops such as tomatoes, peaches and nectarines (Serrano *et al.*, 2004; Gayed *et al.*, 2017). On the other hand, the postharvest application of these substances in controlled amounts is reported to preserve fruit quality in terms of retaining fruit texture, ascorbic acid, high juice yield and prolonged shelf life (Bhattarai and Gautam, 2006; Hussain *et al.*, 2012). Furthermore, calcium chloride minimises respiration and delays senescence in fruit and

vegetables (Akhtar *et al.*, 2010). Dipping of apples (cv. Red Delicious) in calcium chloride solution (0.5 – 2.0 %) alone or combination with gamma irradiation (0.4 k Gy), significantly minimised water loss as compared to untreated fruit at the end of the 90 d storage regime at 2 °C and 90 % RH (Hussain *et al.*, 2012). Similar results are reported in loquat fruit (Akhtar *et al.*, 2010), tomatoes (Bhattarai and Gautam, 2006) and peaches (Gupta *et al.*, 2011). Treatment with calcium and magnesium preserves the functionality of tissues by minimising ion leakage, protein and phospholipid loss (Lester and Grusak, 1999). Calcium promotes strong linkages of pectic substances within the cell-wall (Demarty *et al.*, 1984).

The application of putrescine by pressure-infiltration was observed to significantly minimise water loss in treated pomegranate fruit (cv. Mollar de Elche) to about 13 %, compared to about 17 % in untreated fruit, at the end of the 60 d at 2 °C and 90 % RH (Mirdehghan *et al.*, 2007). Similar results are reported by Barman *et al.* (2011) on ‘Mridula’ cultivar of pomegranate, where fruit treated with putrescine or in combination with carnauba wax significantly minimised water loss as compared to untreated fruit. The results are partly due to the lower respiration rate observed in treated fruit than the untreated fruit as well as the ability of putrescine to minimise chilling injury and maintain tissue integrity (Barman *et al.*, 2011).

Postharvest heat treatment of fruit using hot water and hot air has been reported to significantly enhance the shelf life of fresh produce by minimising chilling injury and decay during storage (Schirra *et al.*, 2011; Huan *et al.*, 2017; Nasef, 2018). Short hot water treatment significantly minimised water loss in cucumbers (cv. Dahshan) (**Table 2.8**). The water loss of cucumbers was observed to decrease with increasing temperature of water from 22.8 % at 25 °C to 16 and 13.6 % at 45 and 55 °C (Nasef, 2018). On the contrary, heat treatment was observed to significantly increase water loss in Satsuma mandarin fruit (cv. Kamei). The hot water treated fruit (52 °C, 120 s) lost about 5.0 % compared to 3.7 % for the control fruit (25 °C, 120 s), after 60 d of storage at 12 – 16 °C and 90 – 95 % (Yun *et al.*, 2013). In our view, this is possible because of the washing away of the protective wax layer and opening up of the clogged surface pores, thus increasing membrane permeability.

Table 2.8 Effect of chemical and physical treatments of fruit water loss during storage

Factor	Fruit	Storage conditions	Application	Water loss (%)	Reference
Chemical	Apples (cv. Red Delicious)	2 °C/90 % RH, 90 d	Untreated (0 % calcium chloride)	5.8	Hussain <i>et al.</i> , 2012
			0.5 % calcium chloride	5.6	
			1.0 % calcium chloride	4.9	
			1.5 % calcium chloride	4.6	
			2.0 % calcium chloride	4.4	
	Loquat (cv. Surkh)	4 °C, 70 d	Untreated (0 % calcium chloride)	3.2	Akhtar <i>et al.</i> , 2010
			1.0 % calcium chloride	3.0	
			2.0 % calcium chloride	2.7	
			3.0 % calcium chloride	2.6	
	Peach (cv. Earli Grade)	0-2 °C/85-90 % RH, 21 d	Untreated (0 % calcium chloride)	5.9	Gupta <i>et al.</i> , 2011
			4 % calcium chloride	5.1	
			6 % calcium chloride	4.7	
Heat	Cucumber (cv. Dahshan)	4 °C/85-90 % RH, 21 d + 4 d (20 °C/80 % RH)	Control (25 °C, 5 min)	22.8	Nasef, 2018
			Hot water (45 °C, 5 min)	16.0	
			Hot water (55 °C, 5 min)	13.6	
Irradiations	Apples (cv. Red Delicious)	2 °C/90 % RH, 90 d	Untreated	5.8	Hussain <i>et al.</i> , 2012
			0.4 k gamma irradiation	4.1	

2.5 Conclusion and prospects

Postharvest water loss during storage, distribution and retail marketing of fresh fruit and vegetables is influenced by various factors in the pre-harvest, harvest and postharvest regimes along the value chain. It is made clear that water loss in biological produce is not simply a measurement, but a complex attribute involving an interplay of multiple morphological, physiological and environmental factors.

Though the fresh fruit industry is more aware of the postharvest factors as emphasised by most researchers, from this literature review it is evident that there are several pre-harvest and harvest factors that can be manipulated to significantly reduce water loss during prolonged storage and marketing. For example, cultivars that are less susceptible to moisture loss can be selected, orchard practices like irrigation schedules and harvest time of the day can be adjusted easily. By taking the necessary control precautions at pre-harvest and harvest stages, the burden of postharvest application of loss control techniques can be minimised, improving profitability in the industry. Major environmental factors such as storage temperature and relative humidity

are of paramount importance, having a great influence on the rate of water loss and the overall quality of fresh produce. The knowledge from this review facilitates the application of a multiscale approach to minimise postharvest water loss along the value chain.

Postharvest water loss varies greatly among fresh produce given a multitude of factors discussed in this review. Interestingly, fruit with tough thick rind such as pomegranate can be more susceptible to postharvest water loss compared to fruit with thin and soft peel such as apples when compared at similar or optimal storage conditions. It is worth noting that there are several uprising challenges associated with the commonly applied water loss control techniques. For example, surface waxing and coating causes the production of off flavours due to anaerobic respiration while the application of plastic liners in cold chain management facilitates moisture condensation and associated fruit decay. Furthermore, the current movement towards a plastic-free packaging (remove/reduce plastics) is a growing challenge in the fruit and vegetable industry. This requires the integral consideration of all the pre-harvest and harvest factors to increase the effectiveness of any alternative packaging system, such as surface coating, in the postharvest to reduce water loss.

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CHAPTER 3

A REVIEW ON THE MECHANISMS AND MODELLING APPROACHES TO WEIGHT LOSS IN FRESH FRUIT

With regard to Chapter 3, pages 55–81, the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Literature search and writing of chapter	80

The following co-authors have contributed to Chapter 3, pages 55–81:

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Declaration with signature in possession of candidate and supervisor	26/02/2020
Signature of candidate	Date

Declaration by co-authors

The undersigned hereby confirm that:

1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 3, pages 55–81,
2. no other authors contributed to Chapter 3, pages 55–81 besides those specified above, and
3. potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 3, pages 55–81 of this dissertation.

Signature	Institutional affiliation	Date
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Chapter 3

3 A review of the mechanisms and modelling approaches to water loss in fresh fruit

Abstract

The physicochemical and sensorial symptoms of fruit water loss are quite obvious including but not limited to shrivelling, changes in product colour, textural properties, total soluble solids, product flavour and saleable weight. However, fruit water loss is a complex process involving the interaction of product morphology, physiology and environmental influence. Fruit are hierarchically structured and have features that extend from molecular scale level to the food plant scale and yet water transport occurs at different spatial scales including nanoscale, microscale, mesoscale and macroscale. Therefore, the modelling of water loss in fresh fruit has been done using micro, macro and multiscale approaches. The objectives of this review are to provide a detailed scientific understanding of the mechanisms of fruit water loss at micro to macro-structural levels and to highlight the approaches applied to model water loss at various spatial scales.

3.1 Introduction

Fruit and vegetables are prone to water loss, which is the major cause of postharvest deterioration (Kays and Paull, 2004; Nunes and Emond, 2007). The symptoms of water loss are quite obvious including but not limited to shrivelling, changes in product colour, textural properties, total soluble solids, product flavour and saleable weight (Maguire *et al.*, 2000; Ben-Yehoshua and Rodov, 2002; Vigneault *et al.*, 2009; Caleb *et al.*, 2013). However, fruit water loss (Maguire *et al.*, 2010; Lufu *et al.*, 2018) is a complex process involving the interaction of product morphology, physiology and environmental influence. Transpiration as the major contributing process to produce mass loss has been demonstrated in tomatoes, pears, pomegranates, strawberries (Shirazi and Cameron, 1993; Xanthopoulos *et al.*, 2014, 2017; Bovi *et al.*, 2018; Lufu *et al.*, 2019). There are other contributing mass loss processes that have been proposed including respiration and mechanisms by which ethylene gas, volatile and aromatic organic compounds are lost, depending on the climacteric or non-climacteric nature of the produce (Amarante, 1998; Olivas and Barbosa-Cánovas, 2005; Vieira *et al.*, 2016; Bovi

et al., 2018). These are often considered negligible given the dominance of the transpiration process.

The majority of the water in food material exits as intra-cellular water within the confines of the cell membrane, cell wall water and inter-cellular water is locked up in the cell wall and intercellular space network, respectively (Karel and Lund, 2003). Depending on transportability, inter-cellular, cell wall and intracellular water can be termed as free, loosely bound and strongly bound water, respectively (Caurie, 2011). Water moves within fresh fruit following different proposed pathways: cell to cell through the interconnected cell wall network (apoplastic pathway), across the cell membrane (*transmembrane* pathway), through the continuous cytoplasmic system (*symplastic* pathway) and intercellular space network (Intercellular pathway) (Taiz and Zeiger, 2002). Furthermore, water movement to the fruit surface occurs by either or combination of convection, molecular diffusion and capillary diffusion mechanisms depending on the driving force responsible for the movement (Datta and Zhang, 1999; Nguyen *et al.*, 2006b). Convective mass transfer, molecular diffusion and capillary diffusion mechanisms are driven by a pressure gradient, concentration gradient and the relative interaction of cohesive and adhesive forces between the liquid and solid phase, respectively (Datta and Zhang, 1999; Nguyen *et al.*, 2006b). At the product surface, water loss is aided and influenced by the existing numerous openings such as stomata, lenticels and micro-cracks and other structures like trichomes. Therefore the interaction of the different driving forces, molecular state, spatial localisation and structural pathways portrays the complexity of transport phenomena in fresh fruit.

With the understanding that fruit are hierarchically structured and have features that extend from molecular scale level to the food plant scale (Ho *et al.*, 2013), water transport has been modelled at different spatial scales including nanoscale (e.g. Aquaporin and cell wall), microscale level (e.g. individual plant cell), mesoscale level (e.g. cortex tissue of an apple fruit) and macroscale level (e.g. whole fruit having different components) (Defraeye, 2014). Recently, the multiscale approach is being applied which combines the details obtained through microscale and mesoscale modelling and applies them at a macroscale level. A multiscale model is a hierarchy of models describing material properties including water transport properties at different spatial scales, in such a way that the underlying sub-models are interconnected (Ho *et al.*, 2013). The authors carried out a detailed review of multiscale modelling. However, a knowledge gap still exists in integrating the different mechanisms of

water loss with the commonly applied water transport models. Thus, this review aimed to discuss the various mechanisms of fruit water loss and the current modelling approach to water loss. The review pays specific attention to the physiological mechanisms of water loss, the micro and macro structure-water loss relations, the micro, macro and multiscale approach to water loss modelling and lastly the role of imaging technologies in transport modelling.

3.2 Physiological mechanisms of water loss

3.2.1 Transpiration

Transpiration is the loss of water from the plant, plant organs like leaves and fruit or vegetables to the immediate surrounding. Mass loss from fresh produce is primarily through transpiration (Kader *et al.*, 1984), a physiological process that continues in fruit and vegetables before and after harvest (Veraverbeke *et al.*, 2003a). Over 97 % of the total mass loss in fresh fruit and vegetables is due to transpiration (Díaz-Pérez *et al.*, 2007). Water follows morphological pathways from the inside of the product, across the surface openings; the stomata, lenticels, bruise damaged areas and cuticle to the surrounding, along a concentration gradient (Veraverbeke *et al.*, 2003c; Thompson *et al.*, 2008).

The rate of moisture loss is dependent on both the driving force and tissue properties. This process is described using Fick's first and second laws of diffusion (Nobel, 2009). According to Fick's first law, the rate at which a product loses moisture is directly proportional to the driving force under steady-state conditions (Nobel, 2009). The driving force responsible for water loss is centred on the differences in the proportion of moisture inside the product and that of the surrounding atmosphere. Therefore, driving force is defined using various parameters that describe the proportionality of moisture in a medium such as partial pressure of water vapour (Ben Yehoshua, 1987; Maguire *et al.*, 2010), water activity (Veraverbeke *et al.*, 2003b), chemical and water potential (Ben-Yehoshua and Rodov, 2002; Veraverbeke *et al.*, 2003a) or simply water concentration in case of passive diffusion (Ben-Yehoshua and Rodov, 2002). In some studies, the driving force in terms of water potential difference was preferred compared to water concentration difference, because the diffusion phenomenon across the produce surface involves water in liquid and vapour states (Veraverbeke *et al.*, 2003c). Knoche *et al.* (2000) further report that water vapour may diffuse across the peel in either liquid or gaseous states. The driving force is majorly influenced by product temperature, environmental temperature and relative humidity (Maguire *et al.*, 2010). Usually, at a particular

product temperature, water vapour is forced to move from the intercellular spaces which have a high water vapour partial pressure (WVP) closer to saturation (Burton, 1982), to the outside air having a lower WVP.

3.2.2 Respiration

Harvested produce continue to live, therefore the energy produced during respiration is used to support cellular biochemical processes (Burton, 1982). Respiration utilises sugars and organic acids in the presence of oxygen to generate energy, produce carbon dioxide, water and dissipated heat (Taiz and Zeiger, 2010). **Figure 3.1** illustrates the concept of the respiration process inside a plant cell showing different mass and heat components involved. Respiration occurs majorly in the mitochondria of the produce cells where the major oxidation enzymes required for the process are found. In many studies, the dependence of respiration rate on oxygen consumption has been demonstrated numerically using Michaelis–Menten kinetics (Hertog *et al.*, 1998; Ho *et al.*, 2010, 2011, 2013). Respiration rate of fresh produce is often expressed in terms of oxygen consumption rate or carbon dioxide production rate. It is important to note that carbon dioxide production rate is one of the indicators of the contribution of respiration in the mass loss of fresh produce. This is because the carbon dioxide gas released outside the produce during gaseous exchange significantly contributes to product mass loss in form of carbon loss. The rate of carbon loss is directly proportional to the respiration rate (Ben-Yehoshua and Rodov, 2002). Furthermore, respiration facilitates and promotes water loss through transpiration mechanism.

Respiration facilitates transpiration in two ways. Firstly, the water generated by substrate oxidation is partially or wholly lost as water vapour during transpiration. Xanthopoulos *et al.* (2017) reported respiratory water loss of up to 39 % in pear fruit stored at 20 °C and 95 % RH, with an average of 8, 14 and 23 % at 0, 10 and 20 °C, respectively. Secondly, the heat that is produced from the respiration process facilitates transpiration in different ways. It is established that the energy produced in the form of adenosine triphosphate (ATP) is used to support cellular life processes and part is lost in the form of heat (Saltveit, 2002). The resulting respiratory heat is used partly as sensible heat increasing product surface temperature (Sastry, 1985; Maguire *et al.*, 2010). This translates into increased water vapour pressure deficit (WVPD) or driving force between the product and its surrounding atmosphere resulting in an increased rate of moisture loss (Maguire *et al.*, 2000). As a result of respiratory heat dissipation, Bovi *et al.* (2018) reported 0.01, 0.07 and 0.12 °C increase in the surface

temperature of strawberries stored at 4, 12 and 20 °C, respectively, under saturated conditions of 100 % RH. Furthermore, a residual transpiration rate of 0.0057, 0.0675 and 0.1737 g kg⁻¹ h⁻¹ was observed in strawberries at the respective storage temperatures. This was attributed to the 0.01, 0.07 and 0.12 °C increase in surface temperature at 4, 12 and 2 °C, respectively, as a result of respiratory heat dissipation (Bovi *et al.*, 2018).

Also, respiratory heat provides the necessary latent heat of vaporisation, resulting in moisture loss to the surrounding atmosphere (Kang and Lee, 1998; Song *et al.*, 2002). For this reason, proper aeration within bulked and stacked produce is recommended as an industrial practice to promote faster cooling process and easy convective removal of dissipated heat during storage and transport. Lowering respiration rate, therefore reduces the rate of moisture loss (Becker and Fricke, 1996). The rate of a respiration process greatly depends on the temperature of the product (Fonseca *et al.*, 2002; Caleb *et al.*, 2012).

Amarante *et al.* (2001) observed that the proportion contributed by respiration to total mass loss varied significantly among pear cultivars. The Comice cultivar had the highest proportion of respiratory mass loss (4.25%), followed by Packharm's (3.86%), Bartlett (2.93%) and Bosc (2.37%) cultivars. These results were despite the fact that the respiration rate was highest in 'Bartlett' pears and more similar among the other cultivars (Amarante, 1998). Furthermore, 'Bartlett' and 'Bosc' had higher carbon dioxide and oxygen permeance than other cultivars. However, because of the higher water permeance of the 'Bosc' and 'Bartlett' pears, the proportion of respiratory mass loss was much lower than in other cultivars (Amarante *et al.*, 2001).

The contribution of respiration rate to overall product mass loss becomes more significant when produce is stored in high humidity environments (low driving force) minimising transpiration process and at high temperatures, increasing respiration (Amarante *et al.*, 2001; Mahajan *et al.*, 2016; Xanthopoulos *et al.*, 2017). Therefore, respiration rate should be put into consideration when evaluating mass loss of products in high humid environments such as inside MAP liners (Ben-Yehoshua and Rodov, 2002). At saturated conditions of 100 % RH where no transpiration is expected, Bovi *et al.* (2018) attributed the residual transpiration rate of 0.006 to 0.174 g kg⁻¹ h⁻¹ to the respiration heat dissipation for strawberries stored at 4, 12 and 20 °C. Respiration contributes about 7 % to total mass loss for apples stored at 0 °C and 90 % RH (Maguire *et al.*, 2000). Furthermore, respiration rate can contribute 9-26 % of total

dry mass loss for pomegranate (cv. Wonderful) stored for 10 weeks at temperatures between 0 – 30 °C (Elyatem and Kader, 1984).

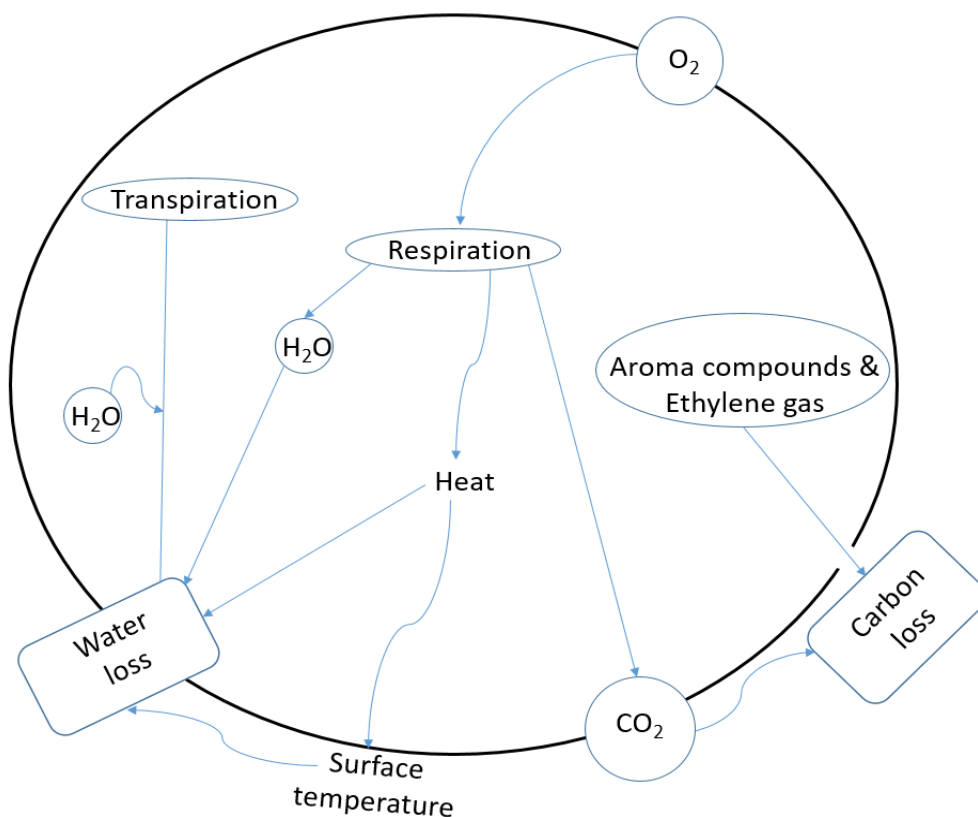


Figure 3.1 The concept of respiration process in a plant cell showing the different mass and heat components involved.

3.2.3 Other mass loss mechanisms

Studies carried out on strawberries have reported the inability of temperature and RH or WVPD to comprehensively account for overall mass loss (Sousa-Gallagher *et al.*, 2013; Bovi *et al.*, 2018). Besides transpiration and respiration, other possible mass flow components that contribute to the total produce mass loss result from mechanisms by which ethylene gas, volatile and aromatic organic compounds are lost, depending on the climacteric or non-climacteric nature of the produce (Amarante, 1998; Olivas and Barbosa-Cánovas, 2005; Vieira *et al.*, 2016; Bovi *et al.*, 2018). The role of these other mechanisms is considered negligible in the mass loss of fresh produce, though their contribution is expected to increase under high humidity storage conditions (Amarante, 1998; Bovi *et al.*, 2018).

3.3 Product structure-water loss relations

3.3.1 Macro-structure

The structures of products that are in the visible range of the naked eye are considered macroscopic ($> \times 10^{-3}$ m). At this level, fruit are made up of several layers of different tissues. A simple fruit consists of three regions: an endocarp region at the centre, mesocarp region and the outer most region (exocarp). The exocarp is further differentiated into the inner layer known as the hypodermis and the outer layer (epidermis). The latter is often overlaid with a non-cellular layer called the cuticle.

Water moves from the inside of the fruit towards the surface following a water concentration or potential gradient and fruit tissues resist water movement to varying degrees. **Table 3.1** shows experimentally determined water transport properties across different fruit tissues. It is observed that the inner tissues such as the mesocarp or cortex have a water diffusivity of two to three magnitudes higher than that in outer tissues of the cuticle (cutin and wax). The cellular layers of the peel epidermis and hypodermis are easily permeated with water owing to their structural composition that involves un-modified cellulose (Burton, 1982). Given that the epidermis and hypodermis offer less resistance to water migration, the cuticle as the outer most non-cellular layer of the peel commonly serves as the last and most important barrier against excessive moisture loss (Holloway, 1982; Knoche *et al.*, 2000). The cuticle consists of cutin layer (composed of cellulose, proteins, phenolic compounds and acids) and a lining of the epicuticular wax layer. It is the waxy part of the cuticle that is responsible for the big resistance against transpiration, majorly due to the hydrocarbons, long-chain alcohols and aldehydes of the wax (Possingham *et al.*, 1967; Veraverbeke *et al.*, 2003a). The wax component contributes 97.9 % of the cuticular diffusive resistance against water conductance as compared to the cutin matrix (2.1 %) (Knoche *et al.*, 2000). The cuticle layer often covers other openings (stomata and lenticels) on the epidermal layer and commonly extends through the epidermal cells in some parts (Konarska, 2013). This, therefore, minimises water loss across the surface openings. Most water conductances were observed across the cuticular membrane (85.7 %) as compared to the stomatal route (14.3 %) in the cheek region of sweet cherry (cv. Sam) (Knoche *et al.*, 2000).

Table 3.1 Moisture diffusivity across fruit tissues

Fruit	Conditions of determination	Part of fruit	Diffusion coefficient ($\text{m}^2 \text{s}^{-1}$)	Reference
Pear (cv. Conference)		Mesocarp (flesh)	1.520E-11 to 1.730E-11	Nguyen <i>et al.</i> , 2003
		Cuticle	1.100E-13 to 1.200E-13	
Pear (cv. Conference)	1 °C	Inner cortex	1.230E-11	Nguyen <i>et al.</i> , 2006b
	20 °C	tissue	4.359E-11	
	1 °C	Outer cortex	5.300E-13	
	20 °C	tissue	1.050E-12	
	1 °C	Cuticle	5.500E-14	
	20 °C		1.280E-13	
Apple (cv. Jonagold)	1 °C/92 % RH	Tissue	1.120E-11	Veraverbeke <i>et al.</i> , 2003c
		Cutin	6.420E-14	
		Wax	2.100E-15	
		Cuticle	8.740E-15	
Apple (cv. Estar)	1 °C/92 % RH	Tissue	4.330E-12	
		Cutin	7.160E-14	
		Wax	3.030E-14	
		Cuticle	5.080E-14	
Apple (cv. Jonagored)	1 °C/92 % RH	Mesocarp tissue	1.380E-11	
		Cutin	4.030E-14	
		Wax	1.920E-15	
		Cuticle	9.100E-15	
Apple (cv. Jonagold)		Flesh	1.030E-10	Verstreken <i>et al.</i> , 1998
		Skin	1.320E-13	

3.3.2 Micro-structure

3.3.2.1 Water pathways inside the fruit

The majority of cells in fresh produce tissues are of the parenchyma type (Mebatsion *et al.*, 2006), possessing thin cell walls and relatively large vacuoles. Water is confined in three regions of the cell. The majority of the water known as intra-cellular water is found within the confines of the cell membrane, while as cell wall water is locked up in the cell wall, and intercellular water is found within the intercellular space network (Karel and Lund, 2003). Depending on transportability, intercellular, intracellular and cell wall water can be termed as free, loosely bound and strongly bound water (Caurie, 2011). This implies that free water is easily lost to the environment, followed by loosely bound and strongly bound water,

respectively. Different pathways have been proposed to describe water movement at the cellular level.

Through the *apoplastic* pathway, water moves from cell to cell through the interconnected cell wall network. Alternatively, water moves through the continuous cytoplasmic system (*symplastic* pathway) from cell to cell across small openings the *plasmodesmata* found in the cell walls of adjacent cells. In the *transmembrane* pathway, water sequentially enters and exits one cell into another across the cell membrane and can cross into the intercellular space (Taiz and Zeiger, 2002). In the Intercellular pathway water moves through the network of intercellular spaces, which is also the major route supporting gaseous exchange between the product and its surrounding. Fresh products consist of loosely bound cells with considerable intercellular spaces, which are interconnected, leading to numerous openings on the product surface. Moisture therefore, diffuses from the cells (regions of high water concentration) into the intercellular spaces (regions of low water concentration) until a near saturation point is reached (Veraverbeke *et al.*, 2003c; Thompson *et al.*, 2008). Furthermore, water diffuses through the intercellular spaces along a concentration gradient from the inner tissues (with high water content) to outer surfaces (with lower moisture content) (Ben-Yehoshua and Rodov, 2002). Water movement along this route occurs both in liquid and vapour states (Veraverbeke *et al.*, 2003a), but predominantly in a liquid state (Burton, 1982), given the minor difference in water vapour deficit between cells of the inside tissues and outside surfaces (Woods, 1990). This is the most widely adopted pathway to explain transpiration in harvested products (Ben-Yehoshua and Rodov, 2002; Veraverbeke *et al.*, 2003c,a).

3.3.2.2 *Water movement to the outside*

At the product surface, water loss is aided and influenced by the existing numerous openings such as stomata, lenticels and micro-cracks and other structures like trichomes. Stomatal density varies among produce, cultivars and surface position on the product. Higher stomatal density was observed on the ventral suture and stylar end than on the cheek regions of sweet cherry (cv. Sam) (Knoche *et al.*, 2000). Stomata tend to lose their functionality in fully developed and harvested produce, because some of the stomata get partially or fully clogged with wax and other materials (Knoche *et al.*, 2000), greatly minimising their role in water transport. However, lenticels are the more likely pores responsible for transpiration and gaseous exchange in harvested mature fruit and vegetables, as compared to stomata

(Veraverbeke *et al.*, 2003c). Lenticels originate from dysfunctional stomata, especially due to skin expansions and when the guard cells lose their ability to control opening and closure during growth and development (Gibert *et al.*, 2010). Just like stomata, some lenticels are clogged with wax or other materials such as suberised periderm, thus providing a barrier against water loss (Veraverbeke *et al.*, 2003a). As a result, the number of open stomata becomes crucial in water loss studies, as observed in apple fruit (cv. Jonagold and Elstar) with an average 42 % ratio of open to closed lenticels (Veraverbeke *et al.*, 2003a,b). Another important outlet of water loss in harvested fruit are the numerous surface micro-cracks. These cracks provide openings for loss of excessive moisture and may facilitate pathogen invasion leading to fruit rotting. In European plums, surface cracks and shrivelling have been identified to co-exist in the regions of high moisture loss occurrences, around the pedicel (Knoche and Peschel, 2007). Surface cracks appear as breakages in the cuticular layer, when the rate at which the product is expanding during development outweighs the rate at which the cuticle layer is deposited to cover the product (Knoche and Peschel, 2007). Therefore, this occurrence is more common in matured or overly matured fruit. Furthermore, high humid conditions result in water redistribution from leaves and branches into the fruit, provoking surface expansion and cracking (Lara *et al.*, 2014).

3.4 Modelling approach to moisture transport

3.4.1 Levels of scale

From a broader perspective, it is not practical to measure all water transport parameters on a very large scale such as factory level or fruit storage facility. However, through modelling, mass transport can be simulated, studied and controlled on a large scale. Moisture loss is the largest contributor to mass loss in fresh produce. Modelling of water transport at different levels of scale including, cellular (micro-structure scale) level, tissue (mesoscale) level, macro-level (whole fruit scale) and multiscale level (bulk and packed fruit) is promoted to facilitate further understanding of the mechanisms of water loss (Fanta *et al.*, 2013). **Table 3.2** shows water transport models in fresh fruit applied at the microscale, mesoscale, macroscale and multiscale.

Table 3.2 Water transport models in fresh fruit applied at the microscale, mesoscale, macroscale and multiscale

Model scale	Fresh produce	Structural/geometrical details	Details of water transport model	Reference
Nano-scale	Artificial plant cell walls		The objective was to study the effect of cell wall composition and temperature on the structure, desorption isotherms and water conductivity of artificial cell walls	Fanta <i>et al.</i> , 2012
Microscale	Pears(cv. Conference)	2D geometric model of cortex tissue, composed of cells of random sizes and shapes, cell walls and intercellular spaces.	A steady-state model applied, Modelling of water transport in the intercellular space, the cell wall network and cytoplasm was done using diffusion laws and irreversible thermodynamics	Fanta <i>et al.</i> , 2013
Microscale	Pear (cv. Conference) cortex tissue intercellular space, cell wall network and cytoplasm	A 2D geometrical model was obtained with a virtual fruit tissue generator, based on cell growth modelling. To account for the microstructure, a microscopic layout was introduced into the modelling as the computational geometry of the model.	Water transport was described using a coupled approach incorporating the microscale water transport model and the cell mechanics model that predicts cell and tissue deformation resulting from hydrostatic stress caused by moisture loss.	Fanta <i>et al.</i> , 2014
Microscale & Mesoscale	Apple (cvs. Jonagold and Elstar) cuticle: Tissue, cutin and wax	Water transport is modelled at a mesoscale (tissue) level by considering moisture loss across the cuticle. However, a more detailed microscopic approach was applied to account for the microstructural features of surface cracks, open and closed lenticels. In the geometrical basis of the moisture diffusion model, lenticel and crack structures,	The actual diffusion properties of the cuticle, cutin and wax were derived from apparent diffusion properties determined experimentally using gravimetric procedures.	Veraverbeke <i>et al.</i> , 2003a

Model scale	Fresh produce	Structural/geometrical details	Details of water transport model	Reference
		total crack area, and effect of wax smoothing were incorporated.		
Mesoscale	Apple fruit tissues (cv. Elstar, Jonagold and Jonagored).	A mesoscale geometry of apple tissue cylinder was constructed to represent fruit tissue, cuticle and cutin and wax layer.	Water transport across fruit tissue, cutin and surface wax layer was simulated. Diffusion coefficients of apple tissue and cuticle were experimentally determined using apple tissue cylinders with intact cuticle, without cuticle or wax.	Veraverbeke <i>et al.</i> , 2003c
Mesoscale	Apple fruit (cv. Jonagold): Cylindrical apple tissue		A coupled mass transfer and mechanics model was used to describe water transport and associated deformation of apple tissue during dehydration. The model was one-dimensional.	Aregawi <i>et al.</i> , 2013
Mesoscale	Conference pears tissues (cuticle, inner and outer cortex)		Water transport in the different fruit tissues was described using sorption isotherm experimental data, fitted with Ferro Fontan model.	Nguyen <i>et al.</i> , 2004
Mesoscale	Conference pears tissues (cuticle, inner and outer cortex)		Water transport in fruit tissues was described based on effective diffusivity of water. Modelling was done using Fick's first and second laws of diffusion on the cuticle and cortex tissues, respectively. A chemical potential gradient was employed as the driving force.	Nguyen <i>et al.</i> , 2006b
Mesoscale and Macroscale	Pears (cv. Conference): cuticle, inner and outer cortex	A computer vision-based modelling system was used. Eight digital images of the fruit taken from different directions. A 3D shape of	The diffusion model was based on Fick's second law, to simulate water transport in whole fruit at shelf (20 °C/75 % RH) and cold storage (1 °C/60 % RH) conditions.	Nguyen <i>et al.</i> , 2006a

Model scale	Fresh produce	Structural/geometrical details	Details of water transport model	Reference
		the whole fruit was reconstructed from contours extracted from the images.		
Macroscale	Apple (cv. Elstar and Jonagold)	A wedge-shaped geometrical model was used to predict moisture loss over a whole apple during 6 months of controlled atmosphere storage.	Different pear tissues with varying diffusion properties were used to describe water transport at a mesoscale level. Actual diffusion coefficients of apple tissue, cutin, wax and cuticle were integrated with different geometric sub-models to predict moisture loss of whole fruit under specific storage conditions.	Veraverbeke <i>et al.</i> , 2003b
Macroscale	Apple whole fruit	Macroscale water transport model for the entire fruit	A microscale model was used to compute the water transport properties of the apple skin. Then the water transport model for the entire fruit was computed from the water transport properties of the skin.	Veraverbeke <i>et al.</i> , 2003c,b
	Macro-scale			
Multiscale (Combined microscale and macroscale)	Apple tissue (cv. Jonagold)		A 2D multiscale water transport and mechanical model was used to predict water loss and viscoelastic deformation. The apparent parameters of the macroscale model were computed from a microscale model. At a microscopic level, water movement across tissue microstructures: cell wall network, cytoplasm and intercellular space were considered.	Aregawi <i>et al.</i> , 2014

3.4.1.1 Nano and Micro-level

This micro level considers the cell as the smallest unit of water transport in fresh produce. At the cellular level, water exists as free (intercellular), loosely bound (intracellular) and strongly bound (cell wall) water, depending on the ease of transportability (Karel and Lund, 2003; Caurie, 2011). As a result, transport models have been developed to account for water movement through cells and intercellular spaces (Fanta *et al.*, 2013). Modelling of water transport at this level appreciates the fact that there is a lot of heterogeneity in tissue structure, resulting from the complexity of different cellular structure and arrangement. This level allows for detailed structural consideration and obtaining physical parameters rather than parameter estimation applied at larger scales of modelling (Ho *et al.*, 2013). The structural complexity at this level can be represented using geometrical models (Marcotte *et al.*, 1991; Fanta *et al.*, 2013).

Given the complexity of cell structure, the micro-scale level can further be broken down to the nano-scale level to give critical attention to structural details of the cell membrane and cell wall. At nano-level, water transport across cell walls has been successfully modelled (Aregawi *et al.*, 2014). The authors applied the unsteady-state diffusion model to describe water transport across the cell walls. Other researchers have coupled water transport models at this level with mechanical deformation models to describe the deformations such as shrinkage, resulting from plasmolysis (loss of turgidity) that can happen in a cell as a result of moisture loss and consequently loss of market quality (Marcotte *et al.*, 1991; Fanta *et al.*, 2014). The water transport properties obtained at this level can further be used in developing larger-scale models of water transport and water loss (Marcotte *et al.*, 1991). However, this demands for tremendously increased computational time and power.

3.4.1.2 Meso-level

Water transport at this level is modelled on plant tissues such as the cortex tissue and cuticular layer of the fruit. Studies in this area have been done on apple and pear fruit, with moisture transport modelled using Fick's first and second diffusion laws (Nguyen *et al.*, 2003, 2006b; Veraverbeke *et al.*, 2003c,a,b). Diffusivity or permeability is the most used parameters to describe water transport and moisture loss at this level and can be deduced from collected experimental data obtained using permeation, sorption-desorption kinetics or moisture concentration techniques (Nguyen *et al.*, 2006b). In the permeation method, diffusivity is obtained from the rate of moisture transfer across the tissue while in sorption-desorption methods, diffusivity is

deduced from sorption-desorption kinetics. On the other hand, diffusivity can be calculated from moisture concentration resulting from diffusion across the axis of cylindrical tissue, using the concentration- distance approach. The permeation and concentration-distance methods have been commonly applied on apple and pear tissues (Nguyen *et al.*, 2003, 2006b; Veraverbeke *et al.*, 2003c), while the desorption method has been applied on tissues of other fruit such as mango slices (Corzo *et al.*, 2008).

3.4.1.3 Macro-level

Water loss of whole intact fruit is commonly modelled based on transpiration process (Mahajan *et al.*, 2008; Caleb *et al.*, 2013; Xanthopoulos *et al.*, 2014, 2017). This is because transpiration is the major contributor to product overall mass loss as compared to the respiration process. Transpiration rate can be expressed using the gravimetric approach, in terms of mass loss of fresh produce (Mahajan *et al.*, 2008; Caleb *et al.*, 2013). The rate of transpiration is directly proportional to the driving force responsible for moisture loss and resistance against moisture loss. This relationship is mathematically expressed as in **Equation 1** and is in line with Fick's first law of diffusion (Sastry and Buffington, 1983; Ben Yehoshua, 1987; Becker and Fricke, 2001; Mahajan *et al.*, 2008; Caleb *et al.*, 2013).

$$TR_m = \kappa_t \cdot (P_s - P_\infty) \quad (1)$$

Where TR_m is transpiration rate on a mass basis ($\text{mg Kg}^{-1} \text{s}^{-1}$); κ_t , transpiration coefficient of a given product ($\text{mg kg}^{-1} \text{s}^{-1} \text{M Pa}^{-1}$); P_s , the water vapour pressure at product surface (M Pa) and P_∞ , the water vapour pressure of the surrounding atmosphere. From **Equation 1**, the driving force ($P_s - P_\infty$) is the vapour pressure deficit between the produce and its surrounding atmosphere, while the inverse of the transpiration coefficient is representing the resistance. The limitation of using transpiration coefficients to calculate transpiration rate (mass loss) is that they are dependent on the product type and operate within a range of experimental conditions (Bovi *et al.*, 2016). Different results can be obtained even for the same product, due to differences in experimental methods applied (Sastry and Buffington, 1983) and the assumptions that are considered in calculating the transpiration coefficient (Bovi *et al.*, 2016).

Furthermore, water permeability has been calculated on whole intact fruit such as in 'Braeburn' apples (Maguire *et al.*, 1999), 'Bartlett', 'Beurre Bosc', 'Doyenne du Comice' and 'Packham's Triumph' pears (Amarante *et al.*, 2001), Japanese plum cultivars 'African Rose', 'Angeleno', 'Ruby Sun', 'Fortune' and 'Ruby Star' (Kritzinger *et al.*, 2018), 'Laetitia' and 'Songgold' plums (Kritzinger and Lötze, 2019) based on Fick's first law of diffusion. However,

the determination of water transport properties of the different tissues is very important in facilitating a detailed investigation of the spatial-temporal moisture distribution within the fruit.

3.4.2 Modelling approaches

Modelling of water transport in fresh fruit has been carried out in two broad approaches: the microscopic approach and the macroscopic continuum approach (Fanta *et al.*, 2013). The latter considers the specimen (material under investigation) as a generalised homogenous entity. As a result, it is a simple and easy way of describing water transport in fruit tissues because it does not necessitate modelling of the microstructures such as pores space, cell membrane and cell wall (Veraverbeke *et al.*, 2003c; Nguyen *et al.*, 2006a,b). In this case, the whole fruit is assumed to be a homogeneous system with general effective properties (Farinu and Baik, 2007; Ikegwu and Ekwu, 2009; Zabalaga *et al.*, 2016). However, detailed insights into water transport at the micro-scale is lost. This is because it employs parameters that are more apparent compared to physical parameters that are attainable at microscopic levels (Ho *et al.*, 2013).

On the other hand, the microscopic approach considers the structural complexity of the material, recognizing the heterogeneity in transport properties existing between compartments. However, a detailed representative geometric construction is relevant. Geometric models of plant tissues have been constructed through imaging of the microstructure using various techniques such as x-ray computed technology (Ho *et al.*, 2014) and microscopy imaging technology (Veraverbeke *et al.*, 2003a). Alternatively, Fanta *et al.* (2013) used a virtual fruit tissue generation algorithm to generate 2D tissue structure composed of randomly sized and shaped cells, cell walls and intercellular spaces. The structure was then compared with fruit tissue micrographs. A change in cell density and volume due to shrinkage was accounted for. The authors then modelled water transport of the intercellular space, the cell wall network and cytoplasm by applying diffusion laws and irreversible thermodynamics according to (Nobel, 2009).

A multiscale approach is one of the recent advances being applied in water transport modelling. This approach has applications in biological sciences where it has been used in a 3D modelling of gas exchange in fruit (Ho *et al.*, 2011; Aregawi *et al.*, 2014). A multiscale model is a hierarchy of models describing material properties including water transport properties at different spatial scales, in such a way that the underlying sub-models are interconnected (Ho *et al.*, 2013). The authors carried out a detailed review on multiscale modelling. Multiscale modelling facilitates computational analysis in solving complex

industrial problems. At this level, we appreciate that foods are hierarchically structured and have features that extend from molecular scale level to the food plant scale (Ho *et al.*, 2013). The different spatial scales involved include nano-level (e.g. Aquaporin and cell wall), microscale level (e.g individual plant cell), mesoscale level (e.g cortex tissue of an apple fruit) and macroscale level (e.g whole fruit having different components) (Defraeye, 2014). On a broader industrial perspective, a mega-scale is introduced considering tonnes of fruit palletised for cold storage and transportation in reefer containers as illustrated in **Figure 3.2**. Multiscale combines the details obtained through microscale and mesoscale modelling and applies them at a macroscale or mega-scale level. The biggest limitation of this level of modelling is large computational time and power required. As a result, averaging procedures are applied to reduce on structural details and obtain more apparent parameter estimates (Ho *et al.*, 2013).

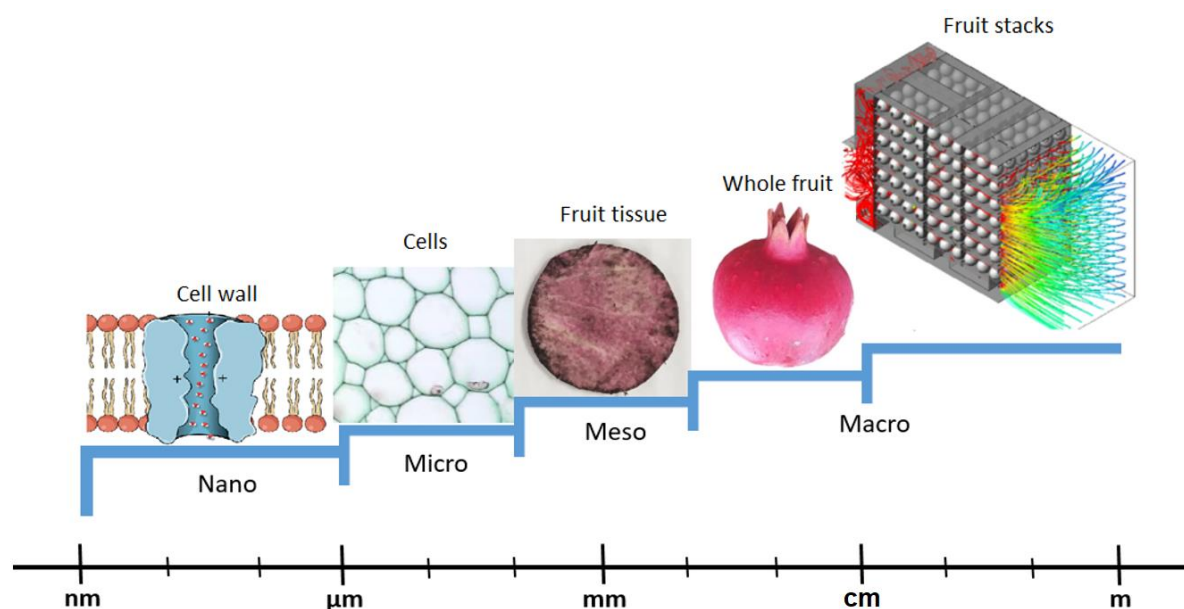


Figure 3.2 Spatial scales of water transport modelling in the fresh fruit industry.

3.5 The role of imaging technologies

One of the ways of improving the accuracy and robustness of water transport models lies in capturing as many details as possible rather than generalising and lumping of important parameters. This is because fruit are highly heterogeneous and complex in structure. Imaging technologies have made it possible to easily visualise the complex structures and subsequently enabled the construction of geometric models required for in-depth simulation studies.

Microscopy technology has been widely exploited to obtain details of fruit surface structures. Common microscopes applied in such studies include confocal laser scanning microscopy (CLSM), scanning electron microscopy (SEM), environmental scanning electron

microscope (ESEM), light microscopy (LM) and transmission electron microscopy (TEM). The combination of two or more of the above microscopy techniques has been applied to examine and characterise the morphology and structure of fruit skin and surface structures such as wax, micro-cracks and stomata in plums (Konarska, 2015; Kritzinger and Lötze, 2019) and apples (Maguire *et al.*, 1999; Veraverbeke *et al.*, 2001; Konarska, 2012; Singh *et al.*, 2016; Yang *et al.*, 2017). A combination of CLSM, ESEM and LM has been used to obtain images of fruit surface layers and structures making it possible to obtain their dimensions (depth, length and width) then the images were used to construct geometric models in the finite element methods (Veraverbeke *et al.*, 2003a). Despite the very high image resolution obtainable by some of these microscopes such as in SEM, the biggest limitation of these microscopy techniques is that they majorly produce data in 2D which is quite limiting in details compared to 3D images.

Technologies such as x-ray computed tomography (X-ray CT) and magnetic resonance imaging (MRI) with the capability of producing 2D images that can easily be reconstructed into 3D images are being sought after. X-ray μ CT has diverse applications in the food industry (Schoeman *et al.*, 2016). This imaging technique creates contrast among fruit structures based on their ability to attenuate X-rays owing to differences in mass densities of the fruit material, with pore space visualised as regions with low intensities. Multiple virtual 2D projection images of slices through the study sample are acquired, followed by reconstruction of consecutive virtual slices to obtain 3D images. The 3D images provide information on pore space distribution making it relevant in simulation studies on heat and mass transport (Ho *et al.*, 2014). Mendoza *et al.* (2007) used X-ray μ CT to visualise and characterise the 3D microstructure and pore space network of apple tissue. Herremans *et al.* (2015) characterised the 3D microstructure of apple (cvs. Jonagold, Kanzi and Braeburn) and pear (cv. Conference) parenchyma tissue, considering individual cells and intercellular spaces. Cantre *et al.* (2014) investigated changes in the 3D microstructure of mango (cv. Carabao) during ripening.

MRI has been embraced as an important non-invasive technique for visualising and monitoring of water transport processes in material science and the food industry. An external magnetic field is applied on the water-containing object, causing the protons (hydrogen nuclei) to become polarised and aligned parallel with the external magnetic field, establishing an equilibrium. Another magnetic field in the form of a radio frequency pulse radiation is applied for a specific time perpendicular to the main magnetic field. This disturbs the previous alignment and the protons produce a rotating magnetic field which is detected as a signal. The

signal undergoes Fourier transformation to produce images which are reconstructed into 3D volume images (McCarthy *et al.*, 1991; Hills, 1995). The intensity of the MRI signal is proportional to the number protons (hydrogen nuclei) in the sample and is often equated to moisture content because the protons are majorly from the water within the sample. Therefore, MRI has been used to acquire temporally and spatially resolved moisture profiles of materials (Bucur, 2003). For example, MRI has been used to detect water core in apples (Wang *et al.*, 1988), estimate parameters of moisture transport in apple tissues (Verstreken *et al.*, 1998), water transfer in meat (Ruiz-Cabrera *et al.*, 2004) and measurement of bound and free water distribution in wood during water uptake and drying (Gezici-Koç *et al.*, 2017).

3.6 Conclusion

It is simple to weigh fruit and determine their mass profile with time. However, the underlying mechanism of water transport phenomenon and water loss are quite not obvious. This review portrays the complexity of water loss in fresh fruit, which involves the combination of physiological and morphological mechanisms. Transpiration (moisture loss) is the major process by which fresh produce lose saleable weight during postharvest handling. Respiration process has been identified as a contributor to physiological mass loss, especially at high relative humidity conditions.

The review details the path of water from the inside of the fruit to the outside, giving specific attention to this phenomenon on the micro and macro scale level. The review acknowledges that fruit are hierarchically structured and have features that extend from molecular scale level (such as cellulose molecule) to whole intact fruit consisting of various tissue types. Therefore moisture transport in fresh fruit can be modelled at different levels of spatial scales including, nano-level (e.g. Aquaporin and cell wall), microscale level (e.g. individual plant cell), mesoscale level (e.g. cortex tissue of an apple fruit) and macroscale level (e.g. whole fruit having different components). However, a multiscale model is recommended which involves a hierarchy of models describing material properties including water transport properties at different spatial scales, in such a way that the underlying sub-models are interconnected.

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SECTION II

Quantitative laboratory experiments to investigate water loss patterns in pomegranate fruit cultivars, the contribution of transpiration and respiration processes to fruit mass loss and determining water transport properties of pomegranate fruit tissues (Chapter 4-6).

CHAPTER 4

CHARACTERISING WATER LOSS AND QUALITY ATTRIBUTES OF POMEGRANATE FRUIT CULTIVARS (‘ACCO’, ‘HERSKAWITZ’ & ‘WONDERFUL’) UNDER COLD AND SHELF STORAGE CONDITIONS

With regard to Chapter 4, pages 85–119, the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Research, data collection and analysis, and writing of chapter	80

The following co-authors have contributed to Chapter 4, pages 85–119:

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Declaration with signature in possession of candidate and supervisor	26/02/2020
Signature of candidate	Date

Declaration by co-authors

The undersigned hereby confirm that:

1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 4, pages 85–119,
2. no other authors contributed to Chapter 4, pages 85–119 besides those specified above, and
3. potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 4, pages 85–119 of this dissertation.

Signature	Institutional affiliation	Date
Declaration with signature in possession of candidate and supervisor	Department of Horticultural Sciences, Stellenbosch University	26/02/2020
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Chapter 4

4 Characterising water loss and quality attributes of pomegranate fruit cultivars ('Acco', 'Herskawitz' & 'Wonderful') under cold and shelf storage conditions

Abstract

Water loss in fresh fruit results in shrivelling and direct financial loss along the supply chain. Particularly, since the peel of pomegranate fruit is highly susceptible to water loss, water loss and shrivelling are crucial in the pomegranate fruit supply chain. However, there is limited knowledge on the mechanism of water loss in pomegranate fruit, given their complex structure. Therefore, the aim of this study was to analyse and characterise water loss in pomegranate fruit. To do this, the physio-chemical properties of two batches of the most important export pomegranate cultivars ('Acco', 'Herskawitz' and 'Wonderful') of South Africa, were monitored, under cold storage and shelf conditions. Batch one was stored for 42 d at 7 °C and 90 % RH and thereafter transferred to shelf conditions of 23 °C and 58 % RH. The second batch was immediately stored under prolonged shelf conditions for 16 d. Water loss, respiration rate, arils-peel proportions and moisture content, peel thickness and colour attributes, puncture resistance property and chemical quality attributes of fruit juice were assessed. Principal component analysis and Pearson's correlation were carried out to assess the variability and to establish relationships among quality parameters. The study revealed that despite the physiological and structural differences among pomegranate cultivars, water loss was similar during the 42 d of cold storage. However, medium-sized fruit ('Herskawitz' and 'Wonderful') had significantly higher water loss ($0.32 \pm 0.01 \text{ g cm}^{-2}$) than small-sized fruit ('Acco') ($0.25 \pm 0.01 \text{ g cm}^{-2}$) during the prolonged 16 d of shelf storage. The study revealed that the maximum water loss of 24.2 % observed in the range of the tested conditions is primarily and majorly from the peel proportion and that peel related properties such as thickness, moisture content and puncture resistance significantly influence water loss. For these findings, we suggest the peel of pomegranate fruit as the key component to be considered in addressing water loss problems.

4.1 Introduction

Pomegranate fruit (*Punica granatum* L.) is a fruit of old, native to the region between Iran and Himalayans in northern India and has over 500 cultivars (Meerts *et al.*, 2009; Chandra *et al.*, 2010). Current global production is estimated at three million tons annually (Erkan and Dogan, 2018) with major cultivation carried out in the Northern Hemisphere and Europe providing the largest global market (CBI, 2019). Though the pomegranate grows favourably under the Mediterranean climate, it has been found to be highly adaptable to various climates and therefore importantly cultivated in the sub-tropical and tropical regions (Roy and Waskar, 1997; Ozgüven and Yilmaz, 2000; Nanda *et al.*, 2001; Waskar, 2011).

The wide knowledge and increasing public awareness about the health benefits associated with pomegranates have tremendously increased consumption (Fawole *et al.*, 2012; Rahmani *et al.*, 2017) especially in the western part of the world. Particularly, there has been a growing demand for high quality, healthy and exotic fruit both for fresh use and for processing into juices and other products (Seeram *et al.*, 2006; CBI, 2019). This has created an opportunity for countries in the Southern Hemisphere including South Africa to export their pomegranates to Europe during the counter off-season in the Northern Hemisphere. Therefore, there is increasing research interest focusing on maintaining pomegranate fruit quality under simulated conditions of harvesting, packaging, transportation, storage and marketing (Elyatem and Kader, 1984; Artés *et al.*, 2000; Waskar, 2011; Arendse *et al.*, 2014).

Pomegranates are highly perishable fruit despite having a relatively lower respiration rate (Barman *et al.*, 2011). Particularly, pomegranate fruit is highly prone to moisture loss due to the plentiful micro-pores and slits in the skin, despite having a thick rind (Nanda *et al.*, 2001; Opara and Al-Ani, 2010; Fawole and Opara, 2013a). Research showed that cultivars such as ‘Bhagwa’, ‘Ruby’ and ‘Wonderful’ can lose 20-25 % of the initial fruit weight within 4 weeks at temperature and relative humidity of 22 °C and 65 % (Fawole and Opara, 2013a; Arendse *et al.*, 2014). During prolonged cold storage, the fruit ‘Bhagwa’, ‘Ruby’ and ‘Wonderful’ lose between 10-16 % of their weight within 12 weeks at 5-7 °C and 90-95 % RH (Fawole and Opara, 2013a; Arendse *et al.*, 2014; Mphahlele *et al.*, 2016; Lufu *et al.*, 2018). A weight loss above 5 % causes shrivelling (Elyatem and Kader, 1984; Lufu, 2017). Even in the absence of any visible shriveling, water loss can undesirably affect the visual appearance, flavour and textural properties of the fruit (Pareek *et*

al., 2015). Excessive water loss results in browning of the peel and arils and hardening of the rind (Kader *et al.*, 1984; Artés *et al.*, 2000; Caleb *et al.*, 2012). It is important noting that pomegranates are considered luxurious fruit that sell well in the higher market segment (CBI, 2019). Therefore water loss can easily cause a huge financial loss to the industry through direct loss of marketable fresh weight and the associated diminished commercial value of affected fruit (Pathare *et al.*, 2013).

Different water loss control techniques have been presented and investigated by many researchers. Storage temperature and relative humidity are important control-parameters (Arendse *et al.*, 2014; Lufu *et al.*, 2019). Plastic liners and modified atmosphere packaging (Caleb *et al.*, 2013; Selcuk and Erkan, 2014, 2015; Banda *et al.*, 2015; Mphahlele *et al.*, 2016; Belay *et al.*, 2018; Lufu *et al.*, 2018), individual shrink wrapping (Nanda *et al.*, 2001; D'Aquino *et al.*, 2010; Mphahlele *et al.*, 2016), waxing and surface coatings (Nanda *et al.*, 2001; Barman *et al.*, 2011; Meighani *et al.*, 2014). These techniques have been applied with great success in minimising the loss of water. However, if not properly used, shrink wrapping and surface coating/waxing can cause anaerobic respiration which leads to the production of off flavours (Gil *et al.*, 1996) while plastic liners facilitate moisture condensation within the bags promoting fruit decay (Lufu *et al.*, 2018). Therefore, there is a need for improvement and design of effective and strategic control techniques. Currently, there is limited knowledge on the characteristics of the water loss of pomegranate fruit, given their complex structure. Hence, the aim of the study was to characterise the water loss of pomegranate fruit based on the fundamental physical and physio-chemical attributes. Secondly, the susceptibility of pomegranate fruit cultivars to water loss was assessed and the contribution of the different parts of the fruit to the water loss was examined. The aim was achieved by monitoring the most important export pomegranate cultivars ('Acco', 'Herskowitz' and 'Wonderful') of South Africa, under cold storage and shelf conditions.

4.2 Materials and methods

4.2.1 Fruit acquisition

Pomegranate fruit (*Punica granatum* L.) of cultivars 'Acco', 'Herskowitz' and 'Wonderful' **Figure 4.1a-c** were harvested at commercial maturity from a commercial orchard situated in Porterville, Wellington (33° 38' S, 19° 00' E), Western Cape Province, South Africa. Fruit were

transported in ventilated plastic trays cushioned with paper pads to the postharvest research laboratory, Stellenbosch University. Sorting of fruit was carried out to ensure size uniformity and that the fruit were free from surface defects such as cracks. Fruit were packed in dozens inside single layer display type paper cartons, cushioned with paper trays at the bottom.

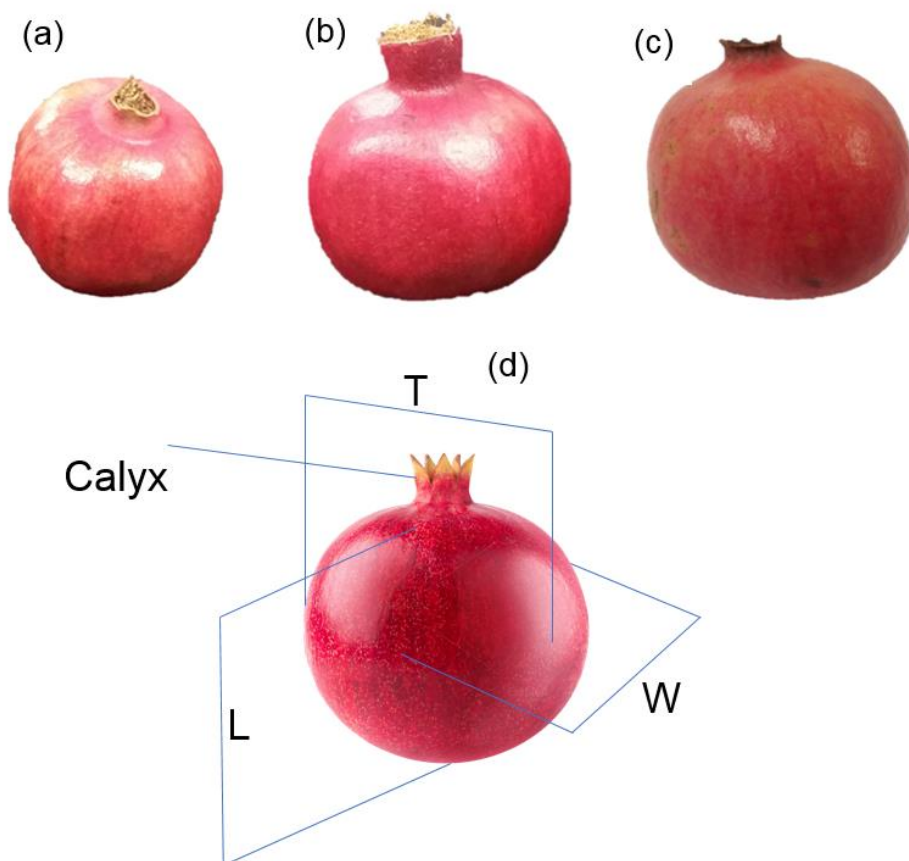


Figure 4.1 The most important pomegranate fruit cultivars grown and exported from South Africa (a) 'Acco', (b) 'Herskawitz' and (c) 'Wonderful'. (d) The basic dimensions of a whole pomegranate fruit (length (L), width (W) and thickness (T)).

4.2.2 Experimental design

A total of 84 fruit (seven cartons) for each of the three cultivars ('Acco', 'Herskawitz' and 'Wonderful') was used in the study. Twelve fruit were used to assess the initial quality of fruit before storage and the remaining 72 fruit were stored in two batches each of 36. Batch 1 fruit were stored at 7 °C and 90 % RH for 42 d and thereafter transferred to shelf conditions of 23 °C and 58 % RH for eight days. This was to mimic the maximum sea freight duration of pomegranate fruit from South Africa to Europe across the Atlantic Ocean, followed by open shelf marketing before

consumption. Twelve fruit were selected for quality assessment after 42 d of cold storage and again after an additional eight days of shelf storage. Batch 2 fruit were immediately stored under shelf conditions of 23 °C and 58 % RH. Then twelve fruit were sampled for quality assessment at the eight and sixteen days of shelf storage. This procedure mimics harvested fruit directly placed under shelf storage without any postharvest treatments.

4.2.3 Measurements

4.2.3.1 Size, weight and colour monitoring

Twelve fruit were randomly selected from each batch and labelled for weight, size and external peel colour monitoring. Measurements were taken before storage and at intervals of seven days throughout the 42 d under cold storage and afterwards at intervals of two days of under the additional shelf period of eight days of Batch 1. For Batch 2, measurements were taken at two days' interval.

The three linear dimensions of the fruit, as shown in (**Figure 4.1d**), were measured using a digital Vernier calliper (Mitutoyo, Kawasaki, Japan, ± 0.01 mm). Fruit length (L) measures the longitudinal dimension (excluding the fruit calyx), while the width (W) and thickness (T) measures the dimensions on the equator (cheeks) of the fruit. Fruit weight was determined using an electronic scientific scale (Mettler Toledo, model ML3002E, Switzerland, 0.0001 g accuracy).

Fruit peel colour was monitored using a digital colourimeter (Minolta, model CR-400, Tokyo, Japan) at the same storage time interval as fruit weight and size. Follow up measurements were carried out at the same marked positions on two opposites sides of each fruit. The lightness (L^*), redness (a^*), yellowness (b^*), hue angle (h°) and chroma (C^*) colour properties were measured according to Commission Internationale de l'Eclairage (CIE), 1976.

A different set of randomly sampled fruit were cut open by hand with the aid of a sharp knife and the arils (edible portion) were separated from the peel. Peel thickness was measured using a pair of digital Vernier callipers (Mitutoyo, model CD-6 CX, Japan) of accuracy 0.01 mm. Opposite peel segments of the fruit were obtained using sharp blades (**Figure 4.2**). Measurements were then taken at the opposite mid-side positions of each segment, obtaining four readings from each of the twelve sampled fruit. The weight of the arils and peels from each individual fruit was

measured using an electronic scientific scale (Mettler Toledo, model ML3002E, Switzerland, 0.0001 g accuracy) to determine their proportions.



Figure 4.2 Pomegranate fruit peel segments showing points of measuring peel thickness (1) along the equatorial axis (2).

4.2.3.2 Headspace gas composition

The headspace gas composition (O_2 and CO_2) was determined using a closed system (Caleb *et al.*, 2012). Two fruit were enclosed in an equilibrated hermetically sealed glass jar, in triplicates, for each storage conditions. Measurements were taken before and after two hours using a calibrated handheld gas headspace analyser (CheckPoint, PBI-Dansensor A/S, Denmark), for separate setups at low temperature (7 °C) and high temperature (23 °C).

4.2.3.3 Fruit firmness

Fruit firmness was determined as puncture resistance (Chen and Opara, 2013; Arendse *et al.*, 2014) with a 5 mm diameter probe (GÜSS-FTA, South Africa). The probe was set to penetrate 8.9 mm into the fruit at 10 mm s^{-1} . The test was carried out on opposite sides of the fruit cheeks, and the peak force (N) required to puncture the fruit was reported as puncture resistance of 24 replicates (2×12 fruit).

4.2.3.4 Moisture content and chemical attributes

Moisture contents of the arils and peel fractions of the fruit were determined by a drying oven method of AOAC 925.45 (AOAC, 2005). The sample was placed at 105 ± 0.5 °C for 24 h in a preheated oven (Model 072160, Prolab Instruments, Sep Sci., South Africa) to achieve a constant weight. The tests were carried out in five replications.

Fresh juice was extracted from the arils using a blender (Mellerware, South Africa). Total soluble solids (TSS) of the fruit juice was measured using a digital refractometer (Atago, Tokyo, Japan). Titratable acidity (TA) of pomegranate juice (PJ) was determined potentiometrically by titration with 0.1N NaOH to an end point of pH 8.2 using a compact auto titrosampler (Metrohm 862, Herisau, Switzerland). Titratable acidity was expressed in milligrams of citric acid (CA) per a hundred millilitres of juice.

4.2.4 Calculations

4.2.4.1 Fruit surface area

Fruit surface area (**Eq 4.1**) was calculated from the fruit geometric mean diameter (**Eq 4.2**) according to Dhineshkumar *et al.* (2015).

$$A = \pi (D_g)^2 \quad (4.1)$$

$$D_g = (L W T)^{1/3} \quad (4.2)$$

Where A (cm²) is the surface area and D_g (cm) is the geometric mean diameter of the fruit, calculated from the length (L (cm)), width (W (cm)) and thickness (T (cm)) of the fruit.

4.2.4.2 Water loss

Cumulative water loss was calculated with respect to the unit fruit mass (**Eq 3**) and with respect to the unit surface area (**Eq 4**) because of the variability in fruit size among cultivars.

$$WL = \frac{(m_i - m_t)}{m_i} \times 100 \quad (4.3)$$

$$WL_A = \frac{(m_i - m_t)}{A} \quad (4.4)$$

Where; WL is water loss per unit fruit mass (%), WL_A is water loss per unit surface area of the fruit (g cm^2), m_i (g) is the initial fruit mass, m_t (g) is the mass of fruit after storage days.

4.2.4.3 Respiration rate

The respiration rate (RR) was calculated in terms of carbon dioxide production rate (R_{CO_2}) in $\text{mL kg}^{-1} \text{h}^{-1}$ by fitting experimentally obtained data into **Eq 5** (Caleb *et al.*, 2012).

$$R_{CO_2} = 10 \times \frac{V_f}{m} \times \left(\frac{C_{CO_{2t}} - C_{CO_{2i}}}{t - t_i} \right) \quad (4.5)$$

Where $C_{CO_{2t}}$ and $C_{CO_{2i}}$ are concentrations (%) of CO_2 at a time t (h) and initial time t_i (h), respectively. In this study $(t - t_i)$ is constant and equals to 2 h. V_f is the free volume (mL) in the jar which is the total volume minus the volume occupied by the fruit and m (g) is the mass of fruit inside the jar, the constant 10 is a unit conversion factor (g kg^{-1}).

4.2.4.4 Colour attributes

The colour intensity describing the length of the colour vector in the a^* - b^* plane was calculated and expressed as chroma (C^*) using **Eq 6** (Pathare *et al.*, 2013) while the hue angle of the colour vectors and the total colour difference (TCD) were given by **Eq 7** and **8**, respectively.

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (4.6)$$

$$h^\circ = \tan^{-1} (b^*/a^*) \quad (4.7)$$

$$TCD = ((L^* - L^*)^2 + (a^* - a^*)^2 + (b^* - b^*)^2)^{1/2} \quad (4.8)$$

Where L^* , a^* and b^* are the reference values and L^* , a^* and b^* are the respective values of lightness, redness and yellowness colour parameters at a given time (Pathare *et al.*, 2013).

4.2.4.5 TSS/TA and BrimA

The balance between sweetness and sourness of the pomegranate juice was estimated in terms of TSS/TA and BrimA which have been reported to influence consumer acceptability (Jordan *et al.*, 2001). BrimA was calculated using **Eq 9** (Jordan *et al.*, 2001).

$$\text{BrimA} = ^\circ\text{Brix} - k \times \text{TA} \quad (4.9)$$

Where k is a constant that ranges from 2 -10, depending on acid and sugar proportions. The k value of 2 was used to avoid a negative BrimA index (Fawole and Opara, 2013a).

4.2.5 Statistical analysis

Measured and calculated data on fruit physical and physio-chemical attributes were analysed using Statistica software (Statistica 14.0, Statsoft, USA). The data was also subjected to analysis of variance (ANOVA) to assess the main effects of cultivar and storage duration. A Duncan's Multiple Range Test was carried to test for statistical significance at $p < 0.05$. Principal component analysis (PCA) and Pearson correlation test were carried out using XLSTAT software (version 2019.1, Addinsoft, France) to assess the variability and to establish relationships among quality parameters.

4.3 Results and discussion

4.3.1 Fruit size and water loss

The three cultivars differed in mass, specific size dimensions along their L , W , T and overall geometric mean diameter D_g (**Table 4.1**). Generally, 'Acco' was a smaller fruit cultivar compared to 'Herskawitz' and 'Wonderful'. It is import to note that fruit size influences the overall surface area to volume ratio and therefore the rate of water loss to the surrounding environment.

The water loss profiles of the three pomegranate fruit cultivars are presented in **Figure 4.3a-b**. Generally, water loss per unit fruit mass (WL) was not significantly different ($P > 0.05$) among the three cultivars during cold storage (**Figure 4.3a**) and the additional days of shelf storage (**Figure 4.3b**), despite higher water loss in 'Herskawitz' than in 'Acco' and 'Wonderful'. In average, water loss reached to 8.74 % at 42 d of cold storage. The subsequent eight days of shelf storage subjected the fruit to an extra 9.04 % water loss. Similarly, there was no significant difference among cultivars during 10 d of direct shelf storage at which water loss reached an average value of 16.8 %. Afterwards, water loss became significantly higher in 'Herskawitz' than in 'Acco' (**Figure 4.3c**). Weight loss was also observed to be cultivar indifferent by (Al-mughrabi *et al.* (1995) between 'Taeifi', 'Banati' and 'Manfaloti' cultivars of pomegranate throughout the cold storage at different temperatures. Furthermore, Fawole and Opara (Fawole and Opara, 2013a)

observed that the weight loss in ‘Ruby’ was relatively similar (20-25 %) to that in ‘Bhagwa’ cultivars of pomegranate fruit after 28 d of shelf storage at 22 °C and 65 % RH.

However, the water loss per unit surface area (WL_A) showed cultivar dependence during the additional shelf storage (**Figure 4.3d-f**). The WL_A was significantly higher in ‘Herskawitz’ than in ‘Acco’ during the additional eight days of shelf storage (**Figure 4.3e**). Similarly, WL_A was significantly lower in fruit cultivars ‘Acco’ than in ‘Herskawitz’ and ‘Wonderful’ during the 16 d of immediate prolonged shelf storage (**Figure 4.3f**).

Table 4.1 Size differences in pomegranate fruit cultivars

Fruit cultivar	Mass (g)	<i>W</i> (cm)	<i>T</i> (cm)	<i>L</i> (cm)	<i>D_g</i> (cm)
Acco	185.71 ± 12.99	73.36 ± 3.18	72.62 ± 3.25	58.67 ± 3.65	67.86 ± 2.94
Herskawitz	302.17 ± 45.37	86.54 ± 7.34	81.33 ± 5.55	76.82 ± 6.51	82.54 ± 5.11
Wonderful	336.32 ± 31.10	90.03 ± 4.24	89.67 ± 4.32	80.91 ± 4.90	86.77 ± 4.20

D_g is the geometric mean diameter a function of the length (L), width (W) and thickness (T) of the fruit. Values are means ± standard deviation of n =12 fruit.

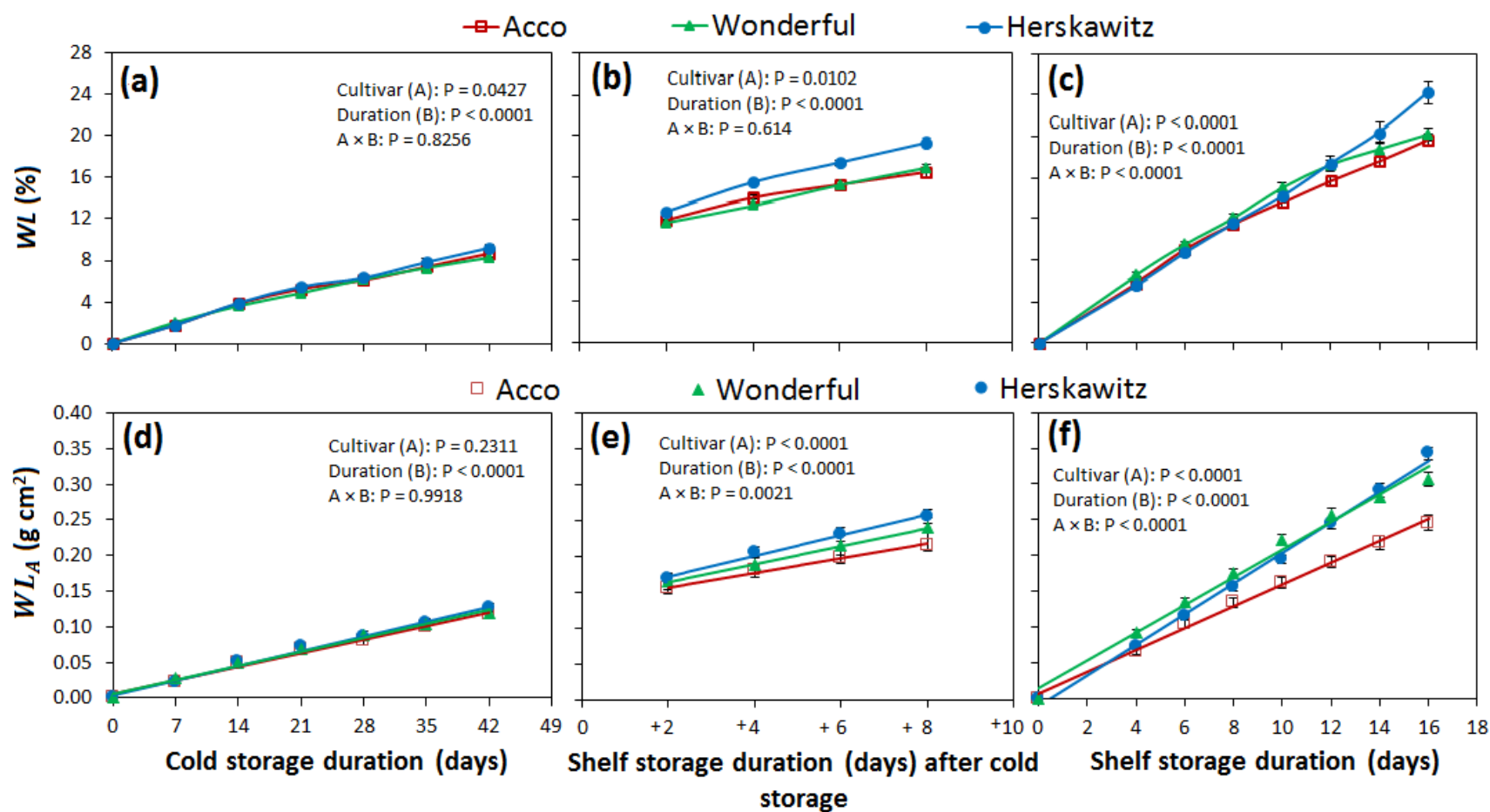


Figure 4.3 Weight loss profile of pomegranate fruit cultivars (‘Acco’, ‘Wonderful’ and ‘Herskawitz’) expressed per unit fruit mass (WL) and per unit surface area (WL_A) during storage: (a and d) for 42 d at 7 °C/90 % RH, (b and e) followed by additional 8 d of shelf storage at 23 °C/58 % RH and (c and f) under immediate prolonged shelf storage of 16 d at 23 °C/58 % RH. The data points are means (n = 12) and the vertical lines represent standard error of the mean. The lines in d, e and f are predictive trend lines fitted on the experimental data. Numerical values of A and B are p-values.

4.3.2 Respiration rate (*RR*)

Figure 4.4 summarises the results on *RR* across all tested conditions. *RR* was significantly ($P < 0.05$) influenced by cultivar across all storage conditions. The *RR* was lowest in ‘Wonderful’ than in other cultivars. At low temperatures (7 °C), a higher *RR* was observed in ‘Acco’ (8.268 mL kg⁻¹ h⁻¹) and ‘Herskawitz’ (6.948 mL kg⁻¹ h⁻¹) than in ‘Wonderful’ (3.289 mL kg⁻¹ h⁻¹) fruit cultivars initially. The change in *RR* was insignificant after 42 d of cold storage at 7 °C and 90 % RH and in the subsequent shelf storage period (**Figure 4.4a**). On the other hand, the effects of cultivar, storage duration, and their interaction significantly influenced the *RR* during the 16 d of shelf life (**Figure 4.4b**). In this case, *RR* at high temperatures (23 °C) decreased from 18.350, 37.936 and 30.612 mL kg⁻¹ h⁻¹ before storage to 9.676, 20.300 and 11.740 mL kg⁻¹ h⁻¹ in ‘Wonderful’, ‘Herskawitz’ and ‘Acco’, respectively, by the end of storage. Other studies have reported a decrease in *RR* of pomegranate fruit with storage duration (Artés *et al.*, 1996) and this could be attributed to progressive senescence of the fruit. A similar situation was observed in a climacteric fruit (pear) stored at different temperature-relative humidity combinations (Xanthopoulos *et al.*, 2017). On the contrary, an increase of *RR* with storage duration has been reported in pomegranate arils, uncoated and coated fruit (Caleb *et al.*, 2012; Meighani *et al.*, 2014).

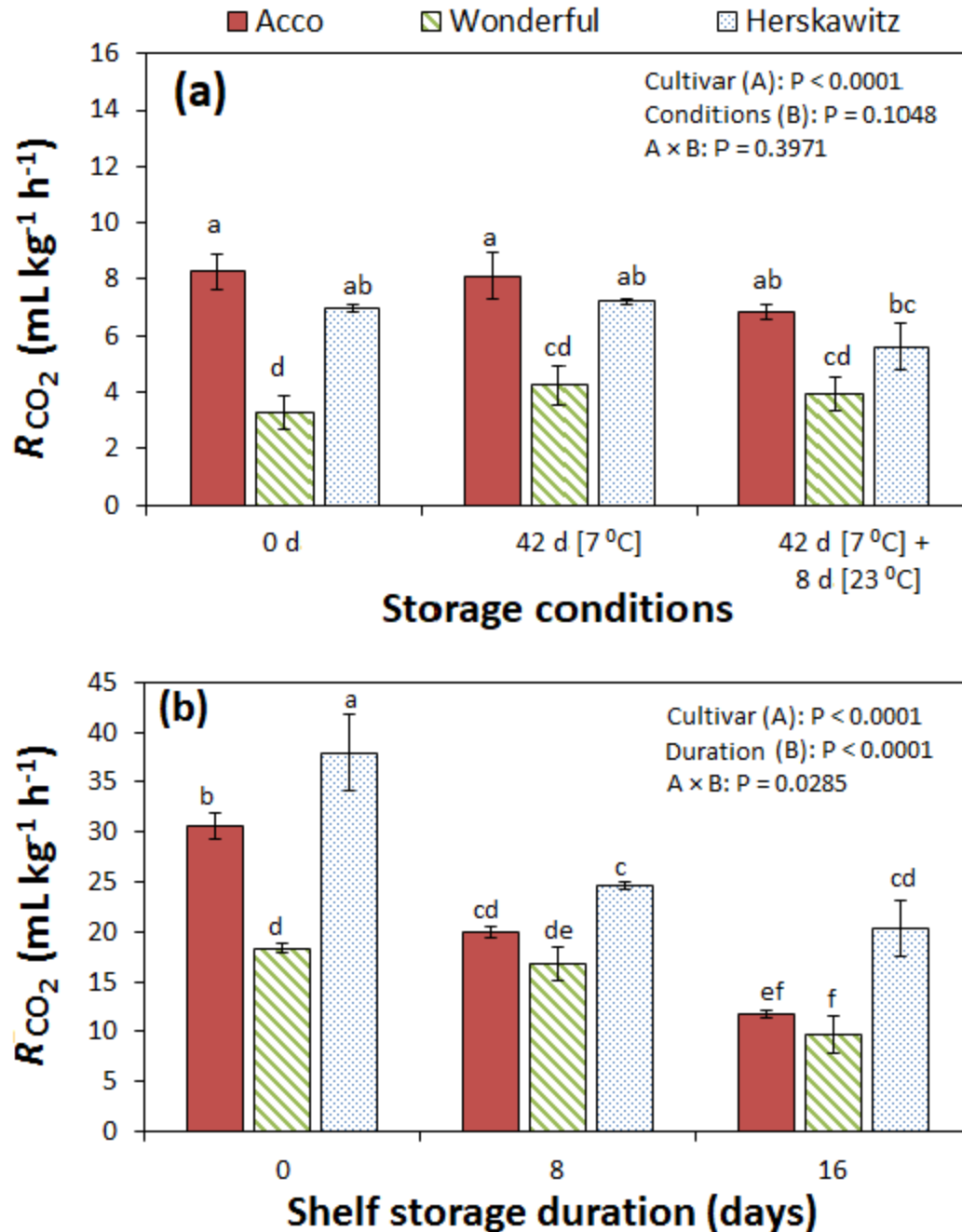


Figure 4.4 Changes in the respiratory carbon dioxide production rate (R_{CO_2}) for pomegranate fruit cultivars ('Acco', 'Wonderful' and 'Herskawitz') during storage: (a) for 42 d at 7 °C/90 % RH followed by additional 8 d of shelf storage at 23 °C/58 % RH and (b) under prolonged immediate shelf storage of 16 d at 23 °C/58 % RH. Measurements taken at 7 °C (a) and 23 °C (b). The bars represent mean values ($n=12$) and the vertical lines are standard errors of the mean. Bars with different letters are significantly different ($P < 0.05$) and numerical values of A and B are p-values.

4.3.3 Peel colour of the three cultivars

Fruit peel colour influences visual appeal and acceptance of pomegranate during marketing (Elyatem and Kader, 1984; Gil *et al.*, 1996). The three cultivars are basically different in peel colour. Generally, all colour parameters were significantly ($P < 0.05$) influenced by cultivar differences and storage duration. Lightness L^* decreased with storage duration under all tested conditions and was significantly lower in ‘Herskawitz’ than in ‘Acco’ and ‘Wonderful’ at the end of fruit storage (**Figure 4.5a-c**). Similarly, peel redness a^* (**Figure 4.5d-f**) and chroma C^* (**Figure 4.6a-c**) decreased with storage duration and were significantly lower in ‘Wonderful’ than in other cultivars. Furthermore, a^* and C^* were more stable with only a slight decrease during the cold storage regime as compared to a steep decline under the shelf life regime. Similar observations were made for the ‘Wonderful’ cultivar by Mukama *et al.* (2019) who observed a continuous reduction in a^* and C^* with storage duration for fruit under low and high relative humidity. The colour change is attributed to the degradation of colour pigments due to water stress (Muche *et al.*, 2018). On the other hand, Arendse *et al.* (2014) observed an initial increase in a^* and C^* for the first 84 d followed by a decrease to the end of the 140 d of storage at 5, 7.5 and 10 °C. The difference in observations among these studies could be attributed to several pre-harvest and harvest factors such as sunlight exposure.

The three cultivars differed in hue angle h° (**Figure 4.6d-f**). Generally, h° was highest in ‘Wonderful’; followed by ‘Acco’ and least in ‘Herskawitz’. Hue angle decreased with storage time, except in ‘Wonderful’ where h° increased after 21 d of cold storage. Total colour difference (*TCD*) between the initial reading and at a given time during storage is presented in **Figure 4.6g-i**. *TCD* significantly and progressively increased with storage duration and was highest in ‘Herskawitz’, followed by ‘Acco’ and least in ‘Wonderful’. This suggests that *TCD* could be used as a good indicator for predicting storage duration for pomegranate fruit under cold and shelf conditions.

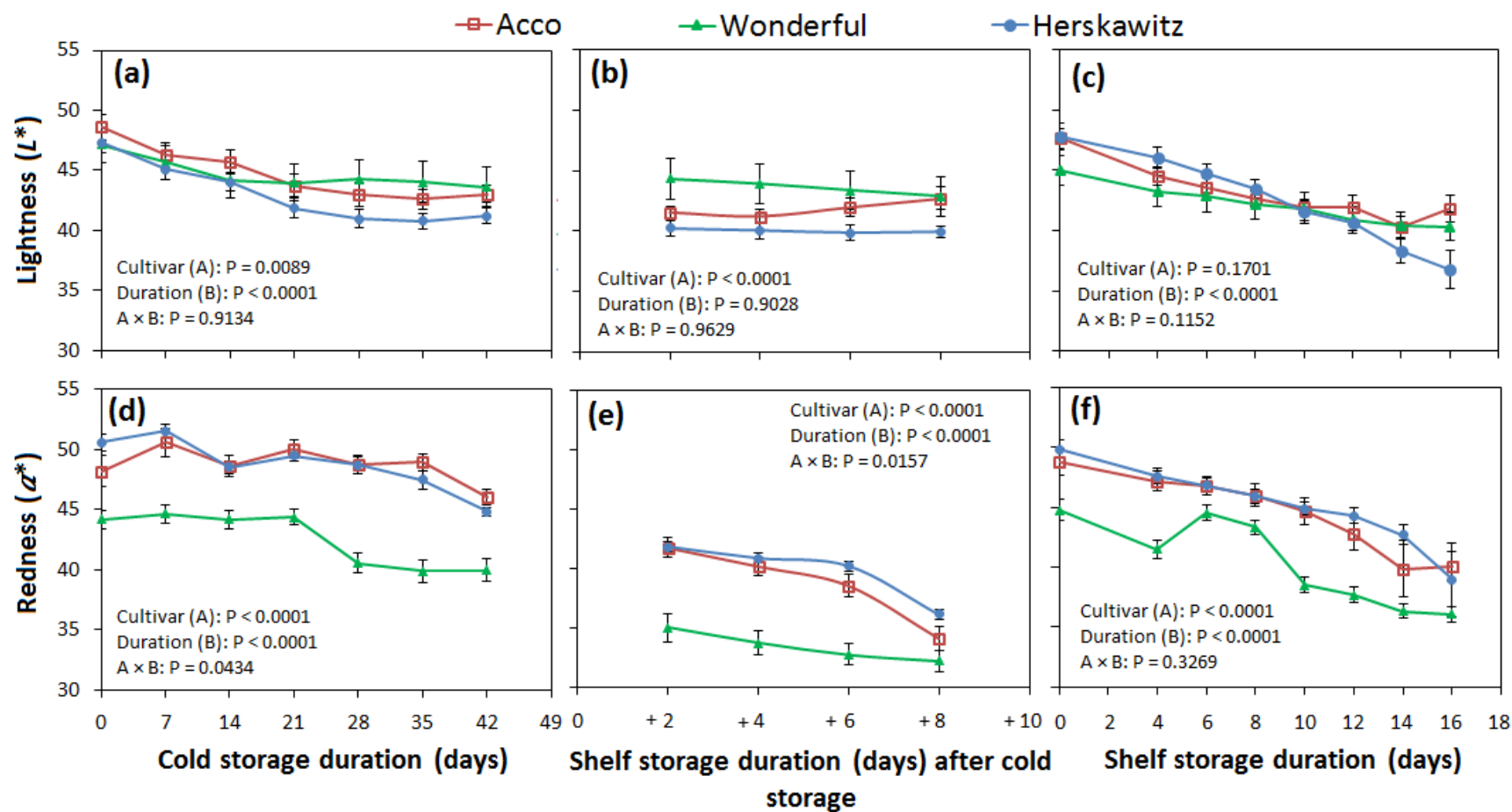


Figure 4.5 Variation of peel colour attributes of lightness (L^*) and redness (a^*) for pomegranate fruit cultivars ('Acco', 'Wonderful' and 'Herskawitz') during storage: (a and d) for 42 d at 7 °C/90 % RH, (b and e) followed by additional 8 d of shelf storage at 23 °C/58 % RH and (b and e) under immediate prolonged shelf storage of 16 d at 23 °C/58 % RH (c and f). The data points represent mean values ($n = 12$) and the vertical lines represent standard error of the mean. Numerical values of A and B are p-values.

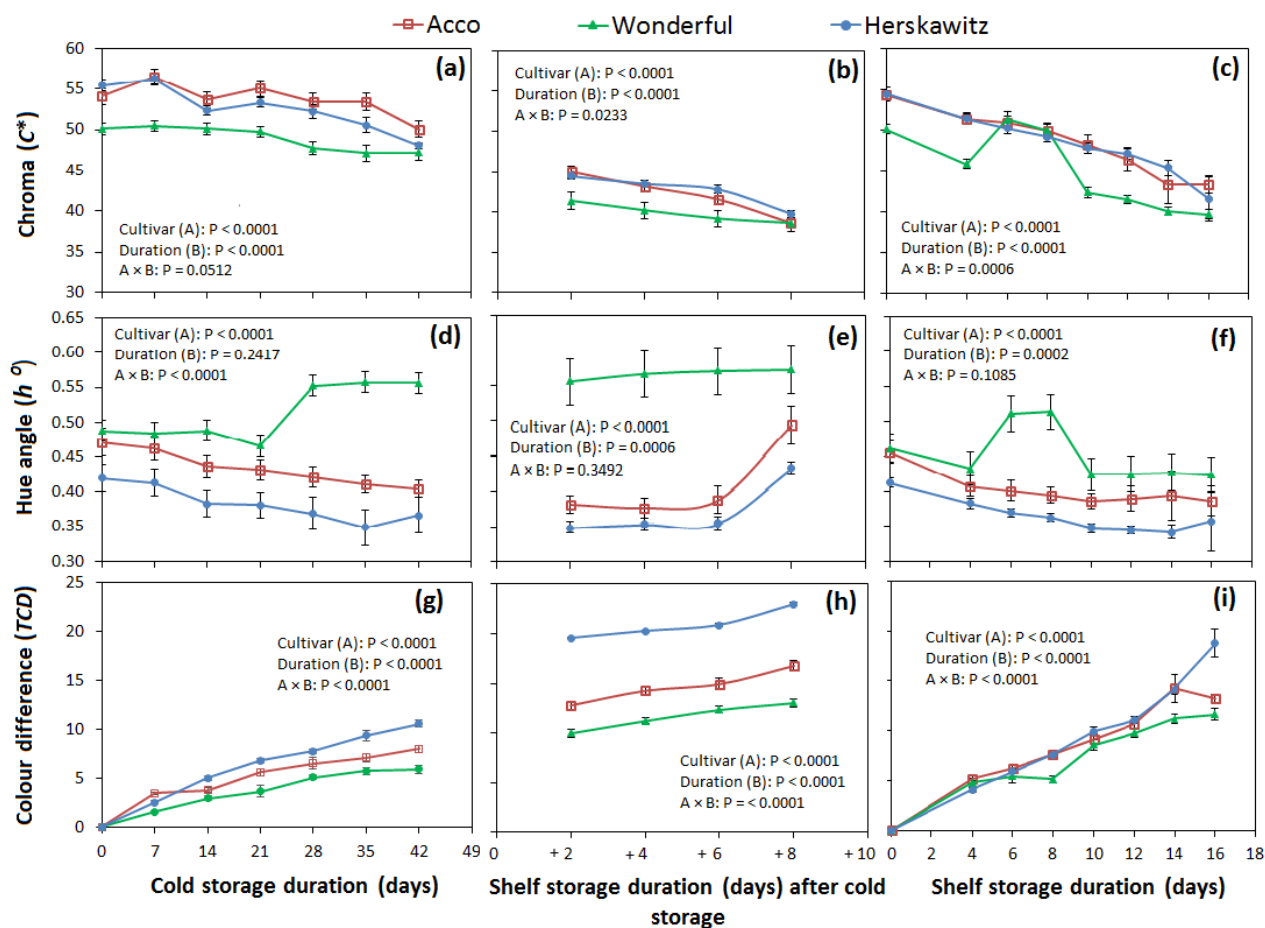


Figure 4.6 Variation in peel chroma intensity (C^*), hue angle (h°) and total colour difference (TCD) for pomegranate fruit cultivars ('Acco', 'Wonderful' and 'Herskawitz') during storage: (a and d) for 42 d at 7 °C/90 % RH, (b and e) followed by additional 8 d of shelf storage at 23 °C/58 % RH and (c and f) under immediate prolonged shelf storage of 16 d at 23 °C/58 % RH (c and f). The data points represent mean values ($n = 12$) and the vertical lines represent standard error of the mean. Numerical values of A and B are p-values.

4.3.4 Fruit firmness

Figure 4.7 shows the results of the fruit firmness test. In general, a significant difference was observed between cultivars. Initially, firmness was significantly lowest in ‘Herskawitz’ (93.04 N) than in ‘Wonderful’ (119.32 N) and ‘Acco’ (131.21 N). The observed difference correlates negatively to the fruit size of the cultivars, with small-sized ‘Acco’ ($Dg = 7.25 \pm 0.27$ cm) having higher firmness than medium-sized ‘Herskawitz’ ($Dg = 8.45 \pm 4.92$ cm) and ‘Wonderful’ ($Dg = 9.23 \pm 0.40$ cm) fruit. These results are buttressed with findings reported by Volz *et al.* (2004) who observed higher firmness in apple fruit (cv. Royal Gala) from the smallest size class than fruit from the largest size class. This was attributed to smaller cells, less air space and greater cell packing in smaller fruit than in larger fruit. An increase in fruit firmness was observed at the end of the 42 d of cold storage followed by a decrease by the end of additional eight days of shelf life especially in ‘Acco’ and ‘Wonderful’ cultivars (**Figure 4.7a**). A quite similar situation (an increase followed by a decrease) is observed for fruit stored immediately at prolonged shelf conditions for 16 d especially for ‘Wonderful’ cultivar (**Figure 4.7b**). Arendse *et al.* (2014) also observed similar behaviour for pomegranate fruit stored under different temperatures (5 – 21 °C) attributing the initial increase in puncture resistance to the decrease in moisture content of the fruit peel resulting into toughening of the peel. The subsequent decline in puncture resistance can be attributed to the observed reduction in peel thickness and senescence of the fruit.

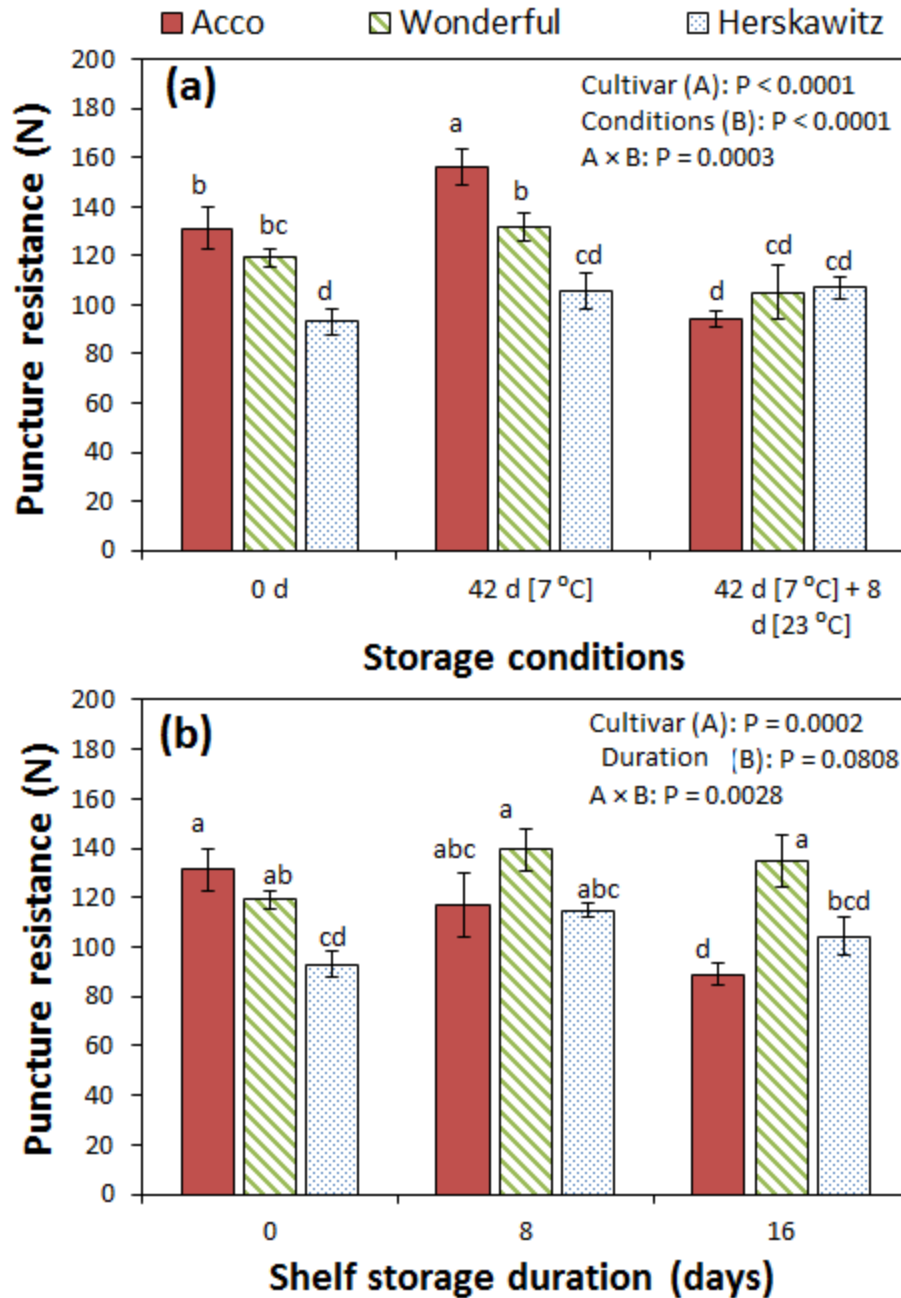


Figure 4.7 Variation in the puncture resistance force for pomegranate fruit cultivars (‘Acco’, ‘Wonderful’ and ‘Herskawitz’) during storage: (a) for 42 d at 7 °C/90 % RH followed by additional 8 d of shelf storage at 23 °C/58 % RH and (b) under prolonged immediate shelf storage of 16 d at 23 °C/58 % RH. The bars represent mean values (n=12) and the vertical lines are standard errors of the mean. Bars with different letters are significantly different ($P < 0.05$) and numerical values of A and B are p-values.

4.3.5 Physical and chemical characteristics of fruit fractions

4.3.5.1 Proportions of fruit fractions

The effect of cultivar, storage duration and their interaction significantly influenced the proportion of fruit fractions. The three cultivars differed in the proportions of arils and peel as summarized in **Figure 4.8**; 59.0 and 41.0 % in ‘Acco’, 50.1 and 49.9 % in ‘Herskawitz’ and 55.4 and 44.6 % in ‘Wonderful’, respectively before storage. However, our results are in close range with the 50.8 – 58.3 % arils and 41.7 – 49.2 % peels reported for other cultivars grown in Oman (Al-Said *et al.*, 2009). Furthermore, the aril proportions in our study are comparable with the 55.6 % in ‘Ruby’ cultivar grown in Morocco (Martínez *et al.*, 2012), 58 % in ‘Ruby’ (Fawole and Opara, 2013b), and 48.5 % in ‘Wonderful’ (Arendse *et al.*, 2016) cultivars grown in South Africa.

Generally, there was no significant increase in the proportion of arils and decrease in the proportion of peel fractions during the 42 d of cold storage (**Figure 4.8a-b**). However, by the end of the additional eight days of shelf life, the proportion of arils fraction significantly increased with the decrease in peel proportion. A more similar scenario was observed during the 16 d of direct shelf life storage, with a higher peel proportion in ‘Herskawitz’ than in other cultivars (**Figure 4.8c-d**). Secondly, the fruit peel proportion decreased with storage time.

Despite having tough thick rind, pomegranate fruit is reported to be more susceptible to weight loss due to the numerous micro-pores on the outer peel (Elyatem and Kader, 1984; Opara and Al-Ani, 2010). The findings of our study further suggest that water loss in pomegranate fruit is primarily and majorly from the peel fraction. Fruit from the ‘Herskawitz’ cultivar had the highest percentage of peel fraction and therefore the highest water loss by the end of each storage regime within the tested conditions. These findings are in agreement with previous research (Nanda *et al.*, 2001; Fawole and Opara, 2013a; Arendse *et al.*, 2014) and are further supported by the results on peel thickness and moisture content analysis reported in the following sections.

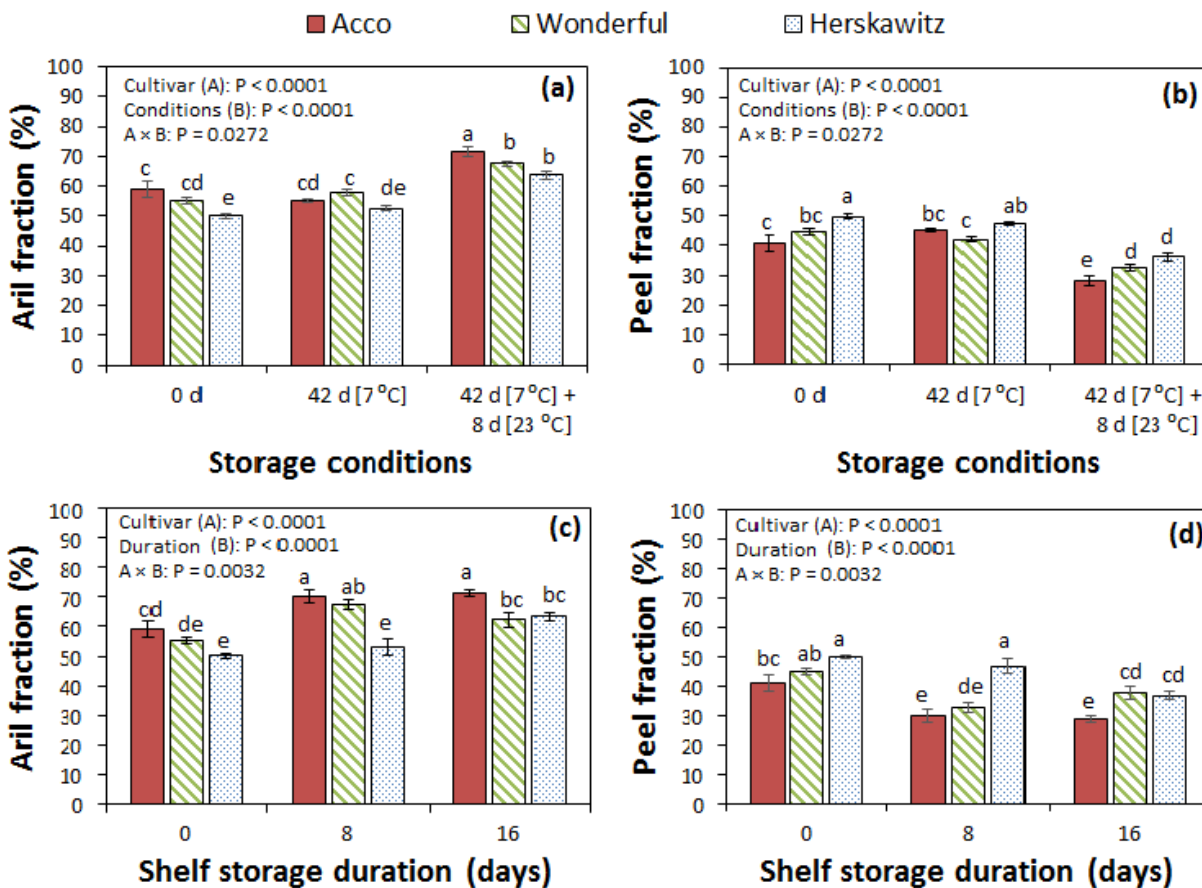


Figure 4.8 Changes in the aril and peel fractions for pomegranate fruit cultivars ('Acco', 'Wonderful' and 'Herskawitz') during storage: (a and b) for 42 d at 7 °C/90 % RH followed by additional 8 d of shelf storage at 23 °C/58 % RH and (c and d) under prolonged immediate shelf storage of 16 d at 23 °C/58 % RH. The bars represent mean values (n=12) and the vertical lines are standard errors of the mean. Bars with different letters are significantly different ($P < 0.05$) and numerical values of A and B are p-values.

4.3.5.2 Peel thickness

Peel thickness measurements initially ranged from 2.66 to 4.38 mm as shown in **Figure 4.9a-b**. These values are in the range (2.68 – 4.70 mm) reported by Al-Said *et al.* (2009) on four pomegranate cultivars grown in Oman. Peel thickness varied significantly among cultivars, greatest in 'Herskawitz' (4.38 mm), followed by 'Wonderful' (4.03 mm) and least in 'Acco' (2.66 mm) initially. Then it decreased with storage duration to 2.13, 1.51 and 1.51 mm, respectively, at the end of the 42 d of cold storage and the additional eight days of shelf storage. Similarly, peel thickness reduced to 1.93, 1.74 and 1.35 mm in 'Herskawitz', 'Wonderful' and 'Acco', respectively after 16 d of absolute shelf storage. The thinning of the peel in time shows that water

loss in pomegranate fruit comes to a great degree from the peel fraction as compared to the aril fraction.

4.3.5.3 Moisture content

The aril moisture content of the three cultivars was similar (81.66 ± 0.99 %) across all conditions of storage while the peel moisture content decreased with storage time (**Figure 4.9c-f**). This clearly indicated that the peel was the main contributor to the water loss of the fruit. Interestingly, the level of water loss observed was not enough to induct any change in the aril moisture content and consequently aril weight. The total water loss of the fruit, in the range of the tested conditions, reached up to 24 %. The observed increase in the proportion of arils in the fruit is due to the reduction in the proportion of the peels. Similar to our results, Arendse *et al.* (2014) reported aril moisture content in the range of 79.74 – 85.11 % with no significant change, for pomegranate fruit (cv. Wonderful grown in South Africa) under prolonged storage for 28 d at 21 °C and 140 d at 10, 7.5 and 5 °C. However, slightly lower aril moisture content (76.01 – 79.09 %) were observed in other cultivars grown in Oman (Al-Said *et al.*, 2009). These differences could be attributed to cultivar and geographical variation.

On the other hand, cultivar and storage duration and their interaction significantly influenced peel moisture content (**Figure 4.9e-f**). Initially, the peel moisture content was greatest in ‘Herskawitz’ (74.29 %), followed by ‘Wonderful’ (71.46 %) and least in ‘Acco’ (63.91 %). Furthermore, peel moisture content decreased with storage time during cold storage and shelf storage. By the end of the 42 d of cold storage plus additional eight days of shelf life, peel moisture content had reduced to 17.18, 30.65, and 36.36 % in ‘Herskawitz’, ‘Wonderful’ and ‘Acco’, respectively.

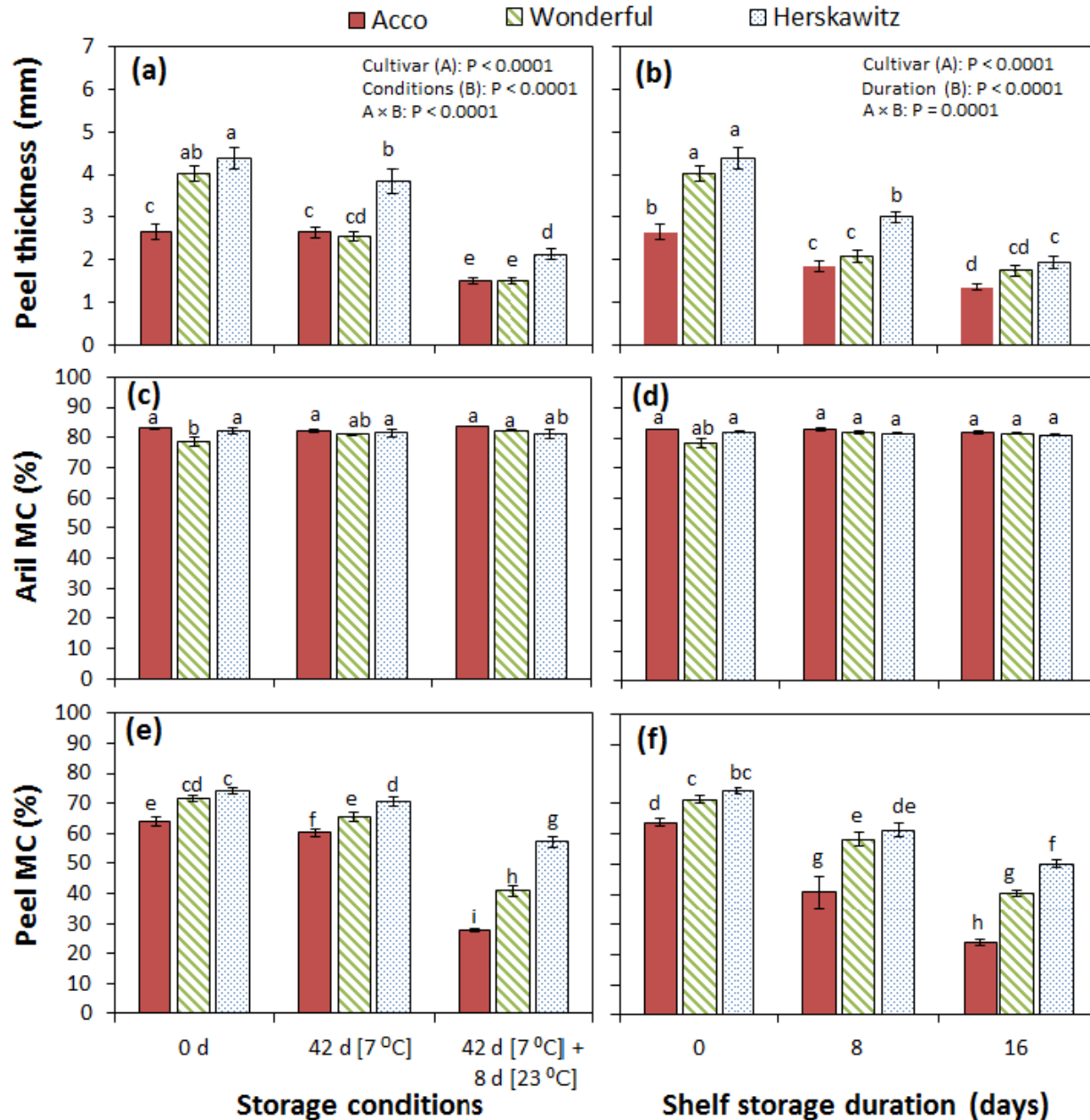


Figure 4.9 Changes in the peel thickness and moisture content (MC) of arils and peel fractions of pomegranate fruit cultivars ('Acco', 'Wonderful' and 'Herskowitz') during storage: (a, c and e) for 42 d at 7 °C/90 % RH followed by additional 8 d of shelf storage at 23 °C/58 % RH and (b, d and f) under prolonged immediate shelf storage of 16 d at 23 °C/58 % RH. The bars represent mean values (n=12) and the vertical lines are standard errors of the mean. Bars with different letters are significantly different (P < 0.05) and numerical values of A and B are p-values.

4.3.5.4 TSS, TA, TSS/TA and BrimA

Chemical attributes of TSS and TA are important in describing the sweetness and sourness of fruit juice taste, respectively (Tandon *et al.*, 2003; Al-Said *et al.*, 2009). The changes in the chemical attributes of the fruit juice with storage time are presented in **Tables 4.2** and **4.3**. Generally, TSS significantly varied among cultivars and was highest in ‘Wonderful’ (16.50 °Brix) than in ‘Acco’ (14.67 °Brix) and ‘Herskawitz’ (14.82 °Brix). A non-significant increase of TSS was observed at the end of the 42 d of cold storage and at the end of the additional eight days of shelf storage (**Table 4.2**). On the other hand, storage duration had a significant influence on the increase in TSS for batch 2 fruit stored immediately under shelf conditions (**Table 4.3**). Our results are comparable with the findings of Mukama *et al.* (2019) who observed a non-significant change in TSS for fruit stored under low RH (65 %) and a significant increase for fruit under high RH (95 %) at 20 °C for 30 d. The authors attributed the increase to the concentration of soluble sugars due to moisture, however, in our particular study we observed no significant change in aril moisture content. Therefore, we attribute the increase in TSS to the hydrolysis of starch and polysaccharides into soluble sugar substrates that are required for utilisation in the respiration process (Díaz-Mula *et al.*, 2009; Valero and Serrano, 2010).

Titratable acidity (TA) was significantly influenced by cultivar effect and the interaction between cultivar and storage time. Before storage TA was lowest in ‘Acco’ (0.25 mg 100 mL⁻¹) than in ‘Herskawitz’ (1.15 mg 100 mL⁻¹) and ‘Wonderful’ (1.62 mg 100 mL⁻¹). TA increased at 42 d of cold storage followed by a decline at an additional eight days of shelf storage, except in ‘Wonderful’ where a consistent decline was observed (**Table 4.2**). It is important to note that TA was significantly lower in ‘Acco’ than in ‘Wonderful’ and ‘Herskawitz’ across all tested conditions. Acco is generally considered as a sweet cultivar as compared to ‘Wonderful’ and ‘Herskawitz’ in the sweet-sour to sour range (Usanmaz *et al.*, 2014). TA remained stable in ‘Herskawitz’ during the 16 d of shelf storage of Batch 2 fruit as compared to a decrease in ‘Wonderful’ and an increase in ‘Acco’ at the end of the storage period. Therefore, different cultivars of fruit responded differently to the storage conditions. Comparably, a decrease in TA has been reported in different cultivars of pomegranates including ‘Wonderful’, ‘Hicrannar’ and ‘Hicaznar’ (Arendse *et al.*, 2014; Selcuk and Erkan, 2014, 2015) under different storage conditions, attributing it to the utilisation of organic acids in metabolic process. On the contrary

Mukama *et al.* (2019) observed an increase in TA for the ‘Wonderful’ cultivar under shelf conditions, attributing it to moisture loss from the fruit.

The effects of cultivar, storage time and their interaction significantly influenced the TSS/TA ratio which was highest in ‘Acco’ than in other cultivars across all tested conditions of storage. TSS/A decreased at 42 d followed by an increase at additional shelf life storage for ‘Acco’ cultivar as compared to the no-significant change in ‘Herskawitz’ and a persistent increase in ‘Wonderful’. On the other hand, TSS /TA decreased in ‘Acco’ cultivar, however, remained stable in ‘Wonderful’ and ‘Herskawitz’ during the 16 d of shelf storage. Generally, fruit with a higher TSS/TA ratio are more preferred by consumers (Boylston *et al.*, 1994).

BrimA index is a variant of TSS/TA ratio that incorporates the tongue’s taste sensitivity (Fawole and Opara, 2013a) and is important in assessing the effect of chemical changes on the flavour (Jordan *et al.*, 2001). BrimA was significantly lower in ‘Acco’ than in other cultivars, however, it was not significantly influenced by storage duration (**Table 4.2**). On the other hand, both cultivar and storage effects significantly influenced BrimA during the 16 d of shelf storage (**Table 4.3**). In this case, BrimA increased with storage time and was generally lower in ‘Herskawitz’ cultivar. Similar results are reported by Arendse *et al.* (2014) for fruits stored under different temperatures.

Table 4.2 Chemical attributes of fruit juice from pomegranate cultivars ('Acco', 'Wonderful' and 'Herskawitz') stored for 42 d at 7 °C/90 % RH followed by additional 8 d of shelf storage at 23 °C/58 % RH

Storage conditions	Cultivar	TSS (°Brix)	TA (mg 100 mL ⁻¹)	TSS/TA	BrimA
0 d	Acco	14.67 ± 0.41 ^b	0.25 ± 0.02 ^f	59.67 ± 4.25 ^a	14.17 ± 0.41 ^{ab}
	Wonderful	16.50 ± 0.21 ^a	1.62 ± 0.04 ^b	10.24 ± 0.28 ^f	13.26 ± 0.22 ^c
	Herskawitz	14.82 ± 0.37 ^b	1.16 ± 0.04 ^c	12.89 ± 0.51 ^{ef}	12.51 ± 0.37 ^d
42 d [7 °C]	Acco	14.99 ± 0.17 ^b	0.58 ± 0.03 ^e	26.60 ± 1.29 ^c	13.84 ± 0.18 ^{bc}
	Wonderful	16.75 ± 0.23 ^a	1.05 ± 0.05 ^c	16.16 ± 0.72 ^{de}	14.64 ± 0.25 ^a
	Herskawitz	14.84 ± 0.27 ^b	1.81 ± 0.10 ^a	8.32 ± 0.46 ^f	11.23 ± 0.29 ^e
42 d [7 °C] + 8 d [23 °C]	Acco	15.12 ± 0.20 ^b	0.48 ± 0.01 ^e	31.89 ± 1.08 ^b	14.16 ± 0.21 ^{ab}
	Wonderful	16.49 ± 0.15 ^a	0.91 ± 0.05 ^d	18.75 ± 1.13 ^d	14.68 ± 0.19 ^a
	Herskawitz	15.30 ± 0.15 ^b	1.56 ± 0.05 ^b	9.91 ± 0.36 ^f	12.19 ± 0.19 ^d
<i>P-values</i>	<i>Cultivar (A)</i>	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	<i>Storage duration (B)</i>	0.3021	< 0.0001	< 0.0001	0.0739
	<i>A × B</i>	0.6294	< 0.0001	< 0.0001	< 0.0001

Table 4.3 Chemical attributes of fruit juice from pomegranate cultivars ('Acco', 'Wonderful' and 'Herskawitz') stored under prolonged immediate shelf storage of 16 d at 23 °C/58 % RH

Storage conditions	Cultivar	TSS (°Brix)	TA (mg 100 mL ⁻¹)	TSS/TA	BrimA
0 d	Acco	14.67 ± 0.41 ^c	0.25 ± 0.02 ^e	59.67 ± 4.25 ^a	14.17 ± 0.41 ^{bcd}
	Wonderful	16.50 ± 0.21 ^b	1.62 ± 0.04 ^b	10.24 ± 0.28 ^c	13.26 ± 0.22 ^{de}
	Herskawitz	14.82 ± 0.37 ^c	1.16 ± 0.04 ^c	12.89 ± 0.51 ^c	12.51 ± 0.37 ^e
8 d [23 °C]	Acco	15.89 ± 0.21 ^b	0.28 ± 0.01 ^e	57.23 ± 2.61 ^a	15.32 ± 0.19 ^a
	Wonderful	17.71 ± 0.18 ^a	1.91 ± 0.17 ^a	9.82 ± 0.76 ^c	13.89 ± 0.41 ^d
	Herskawitz	15.90 ± 0.39 ^b	1.26 ± 0.11 ^c	13.16 ± 0.93 ^c	13.38 ± 0.30 ^{de}
16 d [23 °C]	Acco	16.26 ± 0.12 ^b	0.69 ± 0.08 ^d	24.02 ± 0.98 ^b	14.89 ± 0.15 ^{abc}
	Wonderful	17.47 ± 0.19 ^a	1.25 ± 0.10 ^c	14.77 ± 1.13 ^c	15.02 ± 0.29 ^{ab}
	Herskawitz	16.41 ± 0.23 ^b	1.20 ± 0.08 ^c	14.14 ± 1.00 ^c	14.00 ± 0.22 ^{cd}
<i>P-values</i>	<i>Cultivar (A)</i>	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	<i>Storage duration (B)</i>	< 0.0001	0.1685	< 0.0001	< 0.0001
	<i>A × B</i>	0.5465	< 0.0001	< 0.0001	0.0983

4.3.6 Multivariate analysis

4.3.6.1 Principal component analysis (PCA)

The variability of physical and physiochemical attributes of fruit from the three cultivars was summarised in a PCA. **Figure 4.10a-b** shows that fruit cultivars exhibited both similar and distinct variability in characteristics at different storage periods. The total variability at the different storage conditions was explained by 11 principal factors (F1 to F11) and the first two factors are presented in **Table 4.4**. The first two factors explained 63.3 % of the total variability, with F1 & F2 characterising 39.7 and 23.6 %, respectively. Along F1 of **Figure 4.10a**, postharvest characteristics of ‘Herskawitz’ and ‘Wonderful’ fruit at 42 d of cold storage was to a great extent similar to their characteristics at eight days of shelf storage. This could be attributed to the comparable average water loss of 8.80 % observed in each case. On the other hand, fruit at 42 d of cold storage and an additional eight days of shelf storage is comparable in characteristics with fruit kept for 16 d under immediate shelf conditions. Therefore, pomegranate fruit subjected under enhanced water loss conditions of shelf life, can to a substantial extent be used to make inferences on fruit under prolonged cold storage. Along F2, the characteristics of ‘Acco’ and ‘Herskawitz’ were more on the opposite ends while ‘Wonderful’ was intermediate with a greater leaning towards ‘Acco’ than ‘Herskawitz’.

Along F1, **Figure 4.10b** reveals that of all the tested quality characteristics, fruit at 42 d of cold storage and at eight days of immediate shelf life were more characterised by peel proportion, moisture content and thickness, colour redness (a^*) and intensity (C^*) and fruit puncture resistance. This is supported by high positive factor values under F1 in **Table 4.4**. On the other hand, fruit water loss, water loss per unit surface area and aril proportion can be used to characterise pomegranate fruit at the end of cold storage and additional days of shelf life or fruit under prolonged shelf storage, as supported by the high negative factor values (**Table 4.4**). Along F2, ‘Acco’ fruit (especially at eight days of shelf storage) is characterised by aril moisture content, peel colour lightness (L^*) and hue angle (h°), TSS/TA and BrimA, as depicted by high factor values in F2 (**Table 4.4**). On the other hand, total colour difference (TCD) and titratable acidity (TA) characterise ‘Herskawitz’ fruit especially at the end of both shelf storage regimes.

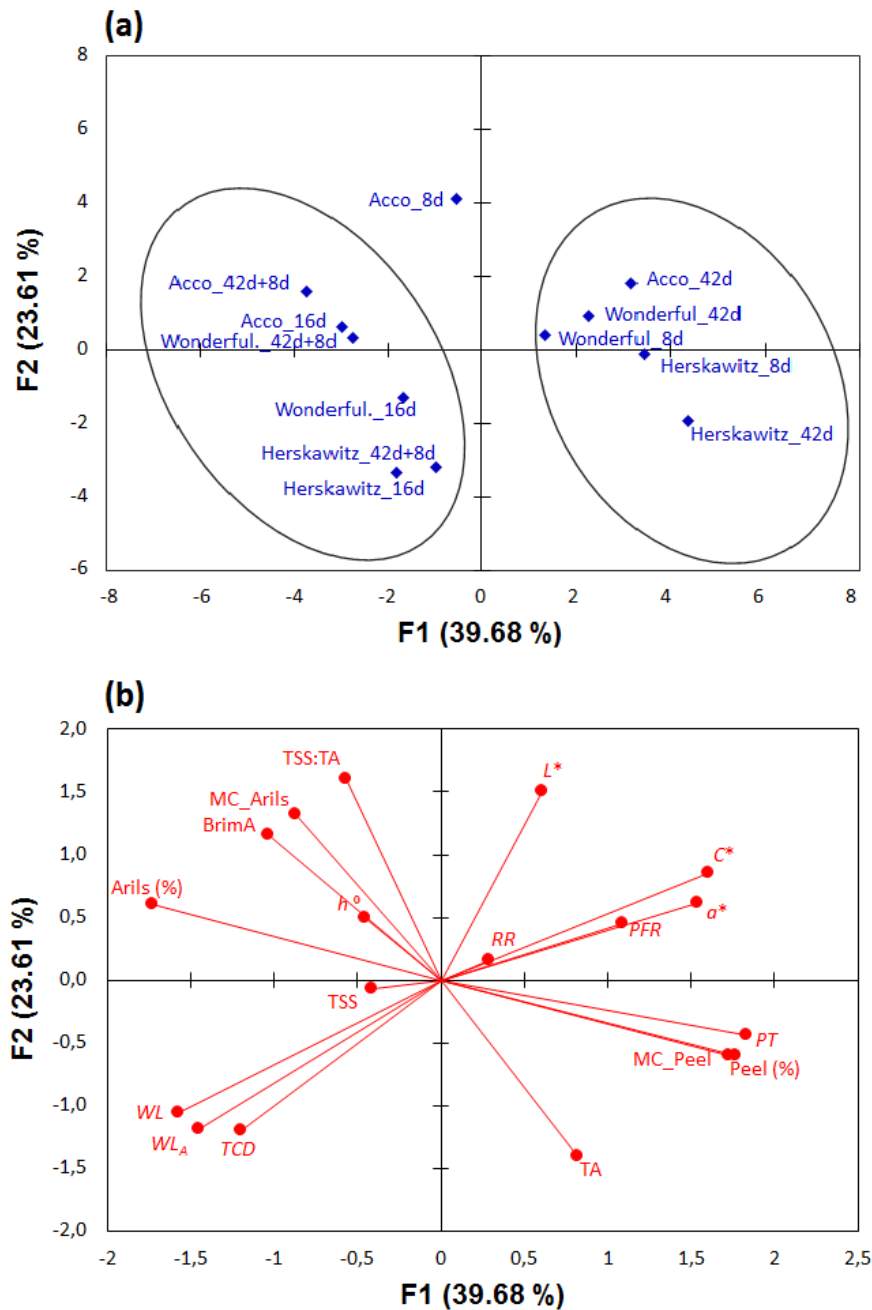


Figure 4.10 Principal component analysis of the first two components (F1 and F2) showing observations (a) and variables (b) based on the physical, mechanical and physio-chemical attributes of pomegranate fruit cultivars (‘Acco’, ‘Wonderful’ and ‘Herskowitz’) stored for 42 d at 7 °C/90 % RH followed by additional 8 d of shelf storage at 23 °C/58 % RH and under prolonged immediate shelf storage of 16 d. WL and WL_A, weight loss per unit mass and unit surface area, respectively; TCD, total colour difference; MC_Arils, moisture content (wet basis) of arils; MC_peel, moisture content (wet basis) of peel; PFR, puncture force resistance; PT, peel thickness;

L^* , lightness; a^* , redness; C^* , chroma; h° , hue angle; TSS, total soluble solids; TA, titratable acidity; RR , respiration rate.

Table 4.4 Factor loadings, scores, eigenvalue and cumulative variance (%) for the first two principal factors (F1–F2) based on the physical and physiochemical attributes of pomegranate fruit cultivars during cold and shelf storage

	F1	F2
<i>Loadings</i>		
Weight loss with respect to fruit mass, WL	-0.792	-0.535
Weight loss with respect to surface area, WL_A	-0.730	-0.598
Arils fraction	-0.868	0.305
Peel fraction	0.868	-0.305
Moisture content of arils, MC_Arils	-0.440	0.667
Moisture content of peel, MC_Peel	0.889	-0.306
Peel thickness, PT	0.922	-0.220
Puncture resistance force, PFR	0.546	0.228
Lightness, L^*	0.305	0.757
Redness, a^*	0.773	0.307
Chroma, C^*	0.806	0.427
Hue angle, h°	-0.229	0.249
Total colour difference, TCD	-0.602	-0.605
Respiration rate, RR	0.147	0.079
Total soluble solids, TSS	-0.208	-0.037
Titratable acidity, TA	0.413	-0.710
TSS/TA	-0.284	0.805
BrimA	-0.522	0.585
<i>Scores</i>		
Acco_42d	3.159	1.806
Acco_42d+8d	-3.741	1.584
Acco_8d	-0.532	4.129
Acco_16d	-3.011	0.643
Herskowitz_42d	4.359	-1.931
Herskowitz_42d+8d	-0.986	-3.176
Herskowitz_8d	3.436	-0.100
Herskowitz_16d	-1.822	-3.329
Wonderful_42d	2.269	0.941
Wonderful_42d+8d	-2.764	0.330
Wonderful_8d	1.304	0.402
Wonderful_16d	-1.670	-1.300
<i>Eigenvalue</i>		
	7.142	4.249
<i>Cumulative variability (%)</i>		
	39.677	63.285

The fruit ('Acco', 'Wonderful' and 'Herskawitz') were stored for 42 d at 7 °C/90 % RH followed by additional 8 d of shelf storage at 23 °C/58 % RH and under prolonged immediate shelf storage of 16 d.

4.3.6.2 Pearson correlation

The Pearson correlation test was performed to establish the relationships among the physical physio-chemical attributes of pomegranate fruit, across all the tested storage condition (**Table 4.5**). Significant ($P < 0.05$) correlations were observed among most of the analysed quality attributes of the pomegranate fruit (**Table 4.5**). Fruit water loss per unit fruit mass WL exhibited strong positive correlations with water loss per unit surface area WL_A ($r = 0.981$) and total colour difference TCD ($r = 0.774$) as well as strong negative correlations with peel thickness PT ($r = -0.647$), peel colour lightness L^* (-0.751), redness ($r = -0.648$) and Chroma intensity C^* ($r = -0.793$). This implies that fruit such as 'Herskawitz' with high water loss per unit surface area and total colour difference are more susceptible to water loss while fruit with high peel lightness, redness and chroma intensity such as 'Acco', are less susceptible to water loss (**Figure 4.10b**).

A significant and moderate negative correlation ($r = -0.508$) exists between water loss WL and peel proportion across all tested conditions. However, this relationship is specifically very strong ($r = -0.823$) for fruit under cold storage and subsequent shelf storage. Furthermore, a significant and very strong positive correlation is observed between peel proportion with peel moisture content ($r = 0.839$) and thickness ($r = 0.892$), as observed in 'Herskawitz' fruit.

Peel chroma intensity is strongly influenced by fruit water loss ($r = -0.793$) and very strongly influenced by redness colour ($r = 0.950$) and positively correlated with fruit puncture resistance (0.531), peel thickness (0.597) and moisture content (0.566).

Other attributes that significantly ($P < 0.05$) correlated with water loss in pomegranates include respiration rate, fruit puncture resistance, peel hue angle, TSS, TSS/TA and BrimA. Respiration rate was significantly and positively correlated with peel redness (0.555). As observed, fruit with higher peel redness ('Acco' and 'Herskawitz') also had significantly higher respiration rate compared to fruit ('Wonderful') with lower peel redness value. Furthermore, the respiration rate was significantly influenced by the TSS as expected while TSS/TA was strongly and negatively influenced by TA ($r = -0.848$) as opposed to TSS ($r = -0.217$). The puncture resistance of the fruit was significantly influenced by the peel moisture content and thickness as expected.

Table 4.5 Pearson correlation coefficient matrix between the physical and physio-chemical attributes of pomegranate fruit (‘Acco’, ‘Wonderful’ and ‘Herskawitz’) stored for 42 d at 7 °C/90 % RH followed by additional 8 d of shelf storage at 23 °C/58 % RH and under prolonged immediate shelf storage of 16 d

Variables	<i>WL</i>	<i>WL_A</i>	Arils (%)	Peel (%)	MC_Arils	MC_Peel	<i>PT</i>	<i>PFR</i>	<i>L</i> *	<i>a</i> *	<i>C</i> *	<i>h</i> ^o	<i>TCD</i>	<i>RR</i>	TSS	TA	TSS:TA	BrimA
<i>WL</i>	1																	
<i>WL_A</i>	0.981	1																
Arils (%)	0.508	0.418	1															
Peel (%)	-0.508	-0.418	-1.000	1														
MC_Arils	-0.066	-0.161	0.574	-0.574	1													
MC_Peel	-0.585	-0.494	-0.839	0.839	-0.637	1												
<i>PT</i>	-0.647	-0.596	-0.892	0.892	-0.415	0.862	1											
<i>PFR</i>	-0.510	-0.394	-0.437	0.437	-0.253	0.505	0.270	1										
<i>L</i> *	-0.751	-0.773	-0.076	0.076	0.413	0.039	0.126	0.254	1									
<i>a</i> *	-0.648	-0.668	-0.499	0.499	-0.172	0.508	0.629	0.412	0.237	1								
<i>C</i> *	-0.793	-0.793	-0.462	0.462	-0.161	0.566	0.597	0.531	0.416	0.950	1							
<i>h</i> ^o	-0.160	-0.105	0.311	-0.311	0.175	-0.067	-0.339	0.148	0.439	-0.540	-0.254	1						
<i>TCD</i>	0.774	0.715	0.311	-0.311	0.035	-0.331	-0.342	-0.606	-0.655	-0.636	-0.791	-0.158	1					
<i>RR</i>	0.071	0.047	-0.023	0.023	-0.124	0.019	0.056	-0.003	-0.183	0.555	0.437	-0.565	-0.238	1				
TSS	0.242	0.344	0.264	-0.264	-0.352	-0.123	-0.404	0.283	-0.102	-0.192	-0.059	0.374	-0.320	0.204	1			
TA	-0.003	0.096	-0.408	0.408	-0.678	0.618	0.486	0.118	-0.330	0.063	0.085	-0.014	0.017	0.018	0.271	1		
TSS:TA	-0.146	-0.253	0.476	-0.476	0.720	-0.497	-0.374	-0.056	0.307	0.184	0.166	-0.072	-0.165	0.189	-0.217	-0.848	1	
BrimA	0.193	0.188	0.561	-0.561	0.310	-0.632	-0.739	0.121	0.205	-0.206	-0.120	0.307	-0.267	0.145	0.553	-0.652	0.563	1

WL and *WL_A*, weight loss per unit mass and unit surface area, respectively; *TCD*, total colour difference; MC_Arils, moisture content (wet basis) of arils; MC_peel, moisture content (wet basis) of peel; *PFR*, puncture force resistance; *PT*, peel thickness; *L**, lightness; *a**, redness; *C**, chroma; *h*^o, hue angle; TSS, total soluble solids; TA, titratable acidity; *RR*, respiration rate. Values in bold are different from 0 with a significance level alpha=0.95.

4.4 Conclusion

The water loss of three pomegranate cultivars ('Acco', 'Wonderful' and 'Herskawitz') was characterised based on the physical and physiochemical attributes of the fruit under cold storage, subsequent shelf storage and immediate prolonged shelf storage conditions.

Generally, the major effect factors: cultivar, storage conditions, and their interaction significantly influenced water loss, respiration rate, peel thickness, peel colour redness and chroma, total colour difference, peel moisture content, fruit firmness and titratable acid. The study reveals that despite physiological and structural differences among pomegranate cultivars, water loss characteristics are similar during the 42 d of cold storage. However, medium-sized fruit ('Herskawitz' and 'Wonderful') are characterised by a relatively higher water loss than small-sized fruit ('Acco') during prolonged storage.

Furthermore, this study revealed that water loss in pomegranate fruit is primarily and majorly from the peel proportion and that peel related properties such as thickness, moisture content and puncture resistance were significantly influenced by the storage duration. Despite the fact that water loss resulted in deterioration of external aesthetic appeal of the fruit, the edible portion of the fruit (the arils) remained unaffected even at total water loss of 24.2 %. Hence, juice yield is minimally affected by storage duration tested. For these findings, we suggest the peel of pomegranate fruit as the key component to be considered in addressing water loss problems. This information is also helpful to plant breeders in selecting against water loss susceptible cultivars.

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CHAPTER 5

THE CONTRIBUTION OF TRANSPIRATION AND RESPIRATION PROCESSES IN THE MASS LOSS OF POMEGRANATE FRUIT

With regard to Chapter 5, pages 122–147, the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Research, data collection and analysis, and writing of chapter	80

The following co-authors have contributed to Chapter 5, pages 122–147:

Name	e-mail address	Nature of contribution	Extent of contribution (%)
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Declaration with signature in possession of candidate and supervisor Signature of candidate	26/02/2020 Date
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Declaration by co-authors

The undersigned hereby confirm that:

1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 5, pages 122–147,
2. no other authors contributed to Chapter 5, pages 122–147 besides those specified above, and
3. potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 5, pages 122–147 of this dissertation.

Signature	Institutional affiliation	Date
Declaration with signature in possession of candidate and supervisor	Department of Horticultural Sciences, Stellenbosch University	26/02/2020
Declaration with signature in possession of candidate and supervisor	Department of Horticultural Sciences, Stellenbosch University	26/02/2020

Chapter 5

5 The contribution of transpiration and respiration processes in the mass loss of pomegranate fruit (cv. Wonderful)

Abstract

Mass loss from fresh produce is associated with quality degradation during storage. Although transpiration is the main process, respiration can significantly contribute to mass loss, especially at higher than normal humidity conditions where the transpiration rate is negligible. In this paper, the relative contribution of the transpiration and respiration processes to the total mass loss of fresh pomegranate fruit were investigated. The study quantified the effects of storage temperature, relative humidity (RH) and time on these processes. Fruit were stored at 5, 15 and 25 °C and 77, 82 and 93 % RH for 43 d. Total rate of mass loss ranged from 0.036 to 0.886 mg m⁻² s⁻¹ (0.002 to 0.060 mg kg⁻¹ s⁻¹). Net transpiration rate ranged from 0.002 to 0.055 mg kg⁻¹ s⁻¹ at vapour pressure deficit (VPD) between 0.010-0.544 kPa. Water loss and carbon losses due to the respiratory process contributed up to 35 % and 23 % at high RH (93 %) and 4 % and 3 % at lower RH (77 %) of the total mass loss, respectively, and the rest was due to VPD. For the range of temperature and RH tested in this study, RH had the greatest influence on the transpiration rate and respiratory mass loss compared to temperature. The relative contribution of the two processes to the total mass loss under different storage conditions is an important step towards improvement and design of effective control strategies.

5.1 Introduction

Fresh horticultural products are widely known for their contribution to micronutrients of human diets worldwide. However, they are highly perishable commodities along the postharvest value chain, from harvest to consumption. These products are highly prone to weight loss due to continued physiological processes (Valero *et al.*, 2015; Caleb *et al.*, 2012b; Maguire *et al.*, 2010), resulting in accelerated loss of quality during storage leading to direct financial loss. The mass loss result from different processes: transpiration, respiration and other processes by which ethylene gas, aromatic and volatile organic compounds may be lost, depending on the nature of the produce (Bovi *et al.*, 2018b).

Pomegranate fruit is botanically considered a berry but its rind (husk) has two parts: an outer, hard pericarp, and an inner spongy mesocarp (commonly referred as albedo) which comprises the fruit inner wall where the arils attach (Stover and Mercure, 2007). Despite the thick leathery rind, a high susceptibility to weight loss has been reported (Nanda *et al.*, 2001; Opara *et al.*, 2010). Fruit weight loss (Maguire *et al.*, 2010; Lufu *et al.*, 2018) is a complex process involving the interplay of product morphology, physiology and environmental influence. It is often referred to and used interchangeably as mass loss (Paull *et al.*, 1997; Maguire *et al.*, 1999), moisture loss (Maw and Mullinix, 2005; Ngcobo *et al.*, 2012; Paniagua *et al.*, 2013) and water loss (Hatfield and Knee, 1988; Harker *et al.*, 2019). From this point on, “mass loss” will be adopted in this study.

Transpiration and respiration are the most contributing physiological processes to mass loss in fruit and vegetables (Elyatem and Kader, 1984; Maguire *et al.*, 2000). These processes promote depletion of water and respiratory substrates, respectively, causing produce mass loss through moisture and carbon dioxide diffusion and heat energy dissipation to the atmosphere (Gaffney *et al.*, 1985; Maguire *et al.*, 2000; Ben-Yehoshua and Rodov, 2002). Transpiration involves the diffusion of water vapour from the intercellular spaces to the product surface and its evaporation to the surrounding environment (Sastry and Buffington, 1983). It is influenced by product-specific factors relating to the morphology such as surface area-mass ratio, and environmental factors such as temperature, relative humidity and airflow velocity (Sastry and Buffington, 1983). Furthermore, it is the greatest contributing process to produce mass loss, especially in tomatoes where transpiration contribution was up to 97 % (Shirazi and Cameron, 1993). Storage studies on apples showed that mass loss mostly comprises water loss (Hatfield and Knee, 1988; Maguire *et al.*, 1999) – a phenomenon driven by transpiration. As a result, previous research using the gravimetric approach or its combination with the theoretical Fick’s diffusion approach, has considered transpiration rate as the rate of change in the total mass of the produce. This has been the case in studies on pomegranate arils (Caleb *et al.*, 2013), mushrooms (Sousa-Gallagher *et al.*, 2013; Mahajan *et al.*, 2016), grape tomatoes (Xanthopoulos *et al.*, 2014), table grapes (Pereira *et al.*, 2017), and strawberries (Sousa-Gallagher *et al.*, 2013).

However, in conditions of high RH and temperature, respiration contributes greatly to mass loss (Gaffney *et al.*, 1985; Kang and Lee, 1998). This is because as the transpiration rate decreases due to high RH, the relative contribution of respiration is occasionally increased. For this reason, current studies are acknowledging the contribution of respiration to water loss and

overall mass loss in fresh produce, rather than considering it negligible. There are three major ways in which the process of aerobic respiration can contribute to overall fruit mass loss: (a) respiratory carbon loss in the form of carbon dioxide gas, (b) respiratory water loss, in which the water generated by substrate oxidation becomes part of the general pool of water lost as water vapour during transpiration, and (c) the respiratory energy produced provides the latent heat necessary for more water vapourisation. Xanthopoulos *et al.* (2017) reported that respiratory water contributes up to 39 % to the total water loss by transpiration for pears of average respiration rate $18.512 \mu\text{g kg}^{-1} \text{s}^{-1}$ stored at 20 °C and 95 % RH. Furthermore, Mahajan *et al.* (2016) acknowledged the contribution of respiratory rate to transpiration water loss of mushrooms, strawberries and red tomatoes by incorporating a representative respiratory heat component to the theoretical Fick's diffusion model. In addition, recent studies have reported that respiratory mass loss as a result of substrate consumption accounted for 81 % of the total mass loss of strawberries stored at 20 °C and 96 % RH (Bovi *et al.*, 2018b). These previous studies only partly consider the contribution of respiration to fruit mass loss in the form of respiratory water loss or respiratory heat or in a more generalised approach as respiratory substrate consumption. Therefore, studies that consider a broader perspective on this subject are very limited. Although the pomegranate is highly susceptible to mass loss during postharvest handling and storage (Artés *et al.*, 2000; Arendse *et al.*, 2015; Opara *et al.*, 2015; Matityahu *et al.*, 2016), the relative contributions of transpiration and respiration processes to the mass loss is still unclear.

The objective of this research was to evaluate the transpiration and respiration characteristics of fresh produce, taking pomegranate fruit as a case study. We investigated the contribution of these processes to overall product mass loss. Furthermore, the influence of storage temperature, RH and storage time on transpiration and respiration were compared. The insights of this research are important in directing the improvement of existing and the design of new strategic mass loss controls in the fresh produce industry.

5.2 Materials and methods

5.2.1 Fruit sample

Pomegranate fruit (*Punica granatum* L., cv. Wonderful) were harvested at commercial maturity from Merwespont farm in Bonnievale (Latitude. -33.9667 °, Longitude. 20.1500 °), Western Cape, South Africa. Fruit were transported in an air-conditioned refrigerated truck to

Postharvest Technology Research Laboratory, Stellenbosch University, and sorted for uniformity of size and elimination of blemished samples. The samples had an average mass of 0.2863 ± 0.0167 kg and a volume of 0.28 ± 0.03 L.

5.2.2 Experimental setup

The salt solutions of sodium chloride, potassium chloride and potassium nitrate were used to simulate an RH of 77 ± 1 , 82 ± 2 and 93 ± 2 %, respectively, inside a 9.5 L container holding two pomegranate fruit (Caleb *et al.*, 2013; Greenspan, 1977) (**Figure 5.1**). The saturated solutions were prepared with analytical grade salts procured from Sigma-Aldrich chemical company (St. Louis, U.S.A) using demineralised water. Saturation was ensured by sonicating the solutions at 25°C for thirty minutes using an ultra-sonic bath (ScieTech, model 705, South Africa) until no more dissolving of crystals. In the course of the experiment, the solutions were strengthened periodically by adding more salt to ensure visible crystals at all time.

Three identically shaped and sized storage rooms (20 m^3 each), equilibrated overnight to an RH of 88 ± 3 % and a temperature of 5, 15 and 25°C were used for the test. Each storage room holds three RH conditions in three replications. Hence, there were a total of 27 containers (9.5 L each holding two pomegranate fruit and saturated salt solution) placed in three storage rooms.

The temperature (T_a) and RH inside each container were monitored using high resolution temperature-humidity data loggers (logTag® HAXO-8, Hong Kong, China) with a measurement range of 0 to 100 ± 0.1 % RH and -40 to $+85 \pm 0.1^{\circ}\text{C}$. The air valve on the lid of the container was opened controllably, to allow for adequate gaseous exchange with the room environment to ensure that the O_2 and CO_2 composition inside the container did not change due to the fruit respiration. This was ensured by monitoring the headspace gas composition inside the containers using a handheld gas analyser (CheckPoint, PBI-Dansensor A/S, Denmark). The setup was found to maintain consistent RH conditions during the experiment.

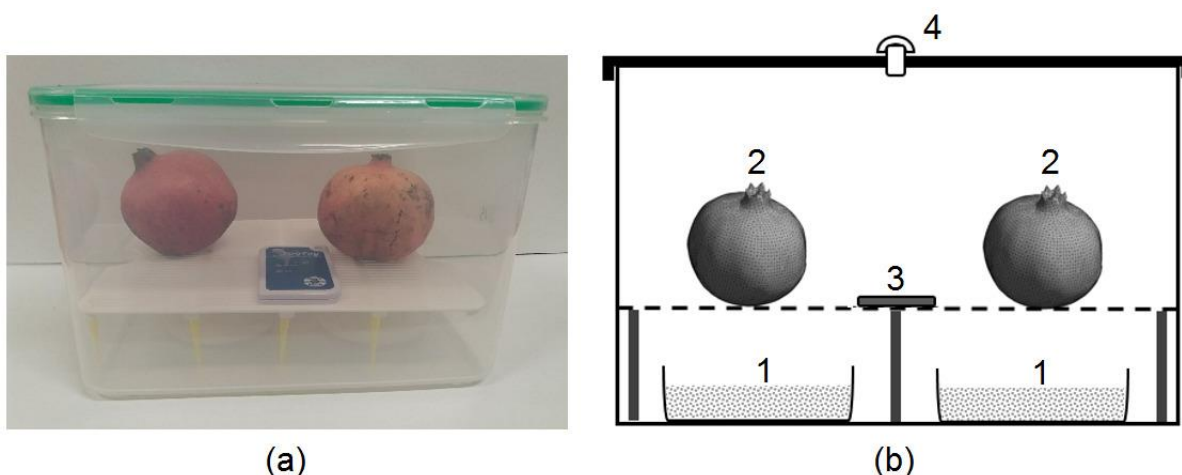


Figure 5.1 Experimental design for monitoring mass loss of pomegranate fruit (cv. Wonderful). Snapshot of the experimental setup (a) and schematics showing the different parts of the setup (b). 1 is the saturated salt solution, 2 is pomegranate fruit samples, 3 is a temperature-humidity data logger and 4 is air valve in the lid of the container.

5.2.3 Measurement procedures

5.2.3.1 Mass monitoring

The fruit were removed from the containers for weight loss measurements every two days for the first eight days. Thereafter, weight loss data was taken at intervals of seven days for thirty-five days. The weight loss is relatively fast at the beginning of the storage period. Hence, a shorter time interval (two days) was implemented at the start for capturing the dynamics of the weight loss accurately. The criteria to stop the storage at 43 d is in consideration of the maximum sea freight duration of pomegranate fruit from South Africa to Europe across the Atlantic Ocean as Europe is the major export destination of South African pomegranate fruit. Mass was measured using a digital scientific scale (Mettler Toledo, model ML3002E, Switzerland, 10 mg accuracy) inside each of the experimental rooms.

5.2.3.2 Fruit size

It is vital to note that the number of fruit per container has an impact on the transpiration rate by influencing the effective surface area and vapour saturation within the container (Bovi *et al.*, 2018b). Fruit surface area to volume ratio influences the rate of moisture diffusion and mass loss (Sastry, 1985), therefore expressing transpiration rate with respect to fruit surface area has practical importance.

To determine the average size of the fruit, three linear dimensions were measured as shown in (**Figure 5.2**). Fruit length (L) was measured at the longitudinal perimeter (excluding

the fruit calyx), while the width (W) and thickness (T) were taken at the equatorial perimeter. The measurements were taken using a digital Vernier calliper (Mitutoyo, Kawasaki, Japan, ± 0.01 mm).

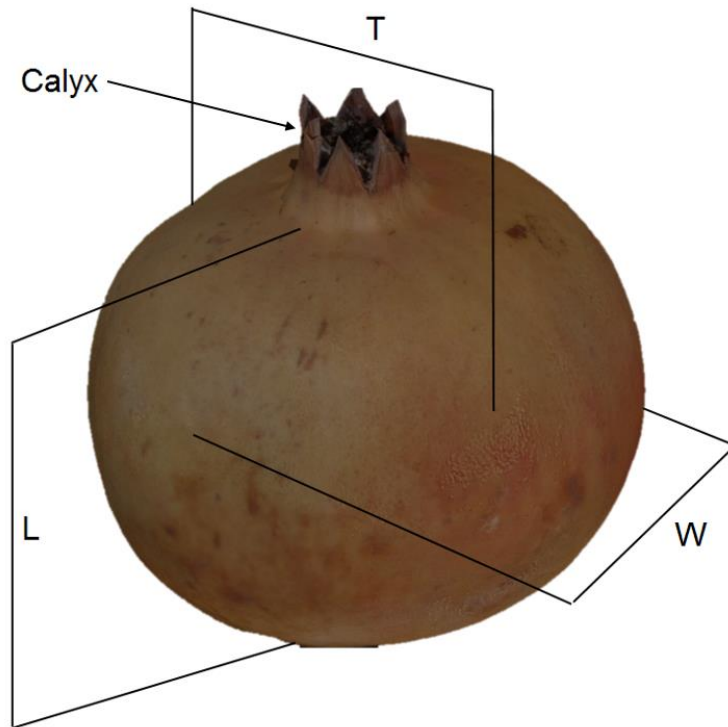


Figure 5.2 Size dimensions of a whole pomegranate fruit including the length (L), width (W) and thickness (T).

5.2.3.3 Fruit respiration rate

The same fruit (monitored for mass loss) were used to determine respiration rate (*RR*) under a closed system (Caleb *et al.*, 2012a). We pre-conditioned the three-litre glass jars for two hours at the respective RH conditions using saturated salt solutions, at 5, 10 and 25 °C. At intervals (four days for eight days and thereafter seven days for thirty five days), the fruit was enclosed in equilibrated hermetically sealed glass jars (two fruit per jar). In triplicates, the jars were stored at the respective temperatures of 5, 10 and 25 °C. The headspace O₂ and CO₂ composition inside jars were analysed before and after two hours using a calibrated handheld gas headspace analyser (CheckPoint, PBI-Dansensor A/S, Denmark) with an accuracy of 0.5 %.

5.2.3.4 Water activity

At the end of the experiment, eighteen fruit (two from each temperature-RH combination) were cut open using hand knives to separate the arils from the white membranes (thin membranous tissue covering the arils) and the peels. Sharp blades were used to separate the exocarp (outer peel fraction) from the mesocarp (inner peel fraction). The water activity (a_w) of the different fruit fractions (arils, white membrane, mesocarp and exocarp) was determined at 5, 15 and 25 °C using a water activity meter (LabMaster-aw, Novasina AG, Switzerland), calibrated in the range of 0.45 – 0.95 water activity using certified humidity standards.

5.2.4 Calculations

5.2.4.1 Estimation of the surface area

The fruit peel provides the surface across which water moves to the surrounding atmosphere. Water loss increases with increasing surface area to mass ratio, therefore expressing transpiration rate per unit surface area of the fruit is justifiable. Fruit surface area was calculated using **Eq. 1** (Dhineshkumar *et al.*, 2015).

$$A = \pi (L W T)^{2/3} \quad (5.1)$$

Where A (m²) is the surface area of fruit, L (m) is the length, W (m) is the width, T (m) is the thickness of the fruit.

5.2.4.2 Vapour-pressure deficit

The saturated vapour pressure was calculated from **Eq. 2** based on the temperature inside the container. Then **Eq. 3** was used to estimate the vapour pressure deficit (VPD) based on the water activity of the fruit surface and the relative humidity of the surrounding air (Maguire *et al.*, 2010).

$$P_s = 0.6108 \times e^{\left(\frac{17.27 \times T_a}{T_a + 273.15}\right)} \quad (5.2)$$

$$VPD = P_s \times (a_w - RH/100) \quad (5.3)$$

Where P_s (kPa) is the saturated vapour pressure at the surrounding air temperature, T_a (°C). The relationship between the transpiration rate and the VPD (kPa) was then established by non-linear regression analysis.

5.2.4.3 Transpiration rate

Transpiration rate was calculated per unit of surface area (TR_A) in $\text{mg m}^{-2} \text{s}^{-1}$ and per unit of initial product mass (TR_m) in $\text{mg kg}^{-1} \text{s}^{-1}$, given by **Eq. 4** and **5**, respectively.

$$TR_A = \frac{(m_i - m_t)}{t \times A} \times 10^6 \quad (5.4)$$

$$TR_m = \frac{(m_i - m_t)}{t \times m_i} \times 10^6 \quad (5.5)$$

Where; m_i (kg) is the initial fruit mass, m_t (kg) is the mass of fruit at time t (s) and A (m^2) is the surface area of the fruit.

5.2.4.4 Respiration rate

The respiration rate (RR) was calculated in terms of carbon dioxide production rate (R_{CO_2}) in $\mu\text{g kg}^{-1} \text{s}^{-1}$ by fitting experimentally obtained data into **Eq. 6** (Caleb *et al.*, 2012a), assuming negligible dissolved CO_2 within the fruit fluids.

$$R_{CO_2} = \rho V_f / m_t \times \left(\frac{C_{t CO_2} - C_{i CO_2}}{t - t_i} \right) \times 10^7 \quad (5.6)$$

Where $C_{t CO_2}$ and $C_{i CO_2}$ are concentrations (%) of CO_2 at a given time t (s) and initial time t_i (s), respectively. ρ (kg L^{-1}) is the density of CO_2 gas and V_f (L) is the free volume in the jar which is the total volume minus the volume occupied by the fruit and saturated salt solution.

5.2.4.5 Estimation of mass loss due to respiration

The mass loss due to respiration was estimated using the stoichiometric equation of the respiration process based on the measured carbon dioxide production rate (R_{CO_2}). This approach is based on the assumptions of a) stable aerobic respiration conditions b) glucose and fructose which are the major sugars in pomegranate fruit juice (Miguel *et al.*, 2004; Dafny-Yalin *et al.*, 2010) as the oxidation substrates and c) all the water produced during respiration was lost as water vapour through transpiration process. The approach has been applied in different studies that acknowledge the contribution of respiration to water loss in fresh produce (Becker *et al.*, 1995; Kang and Lee, 1998; Song *et al.*, 2002; Gross *et al.*, 2016; Xanthopoulos *et al.*, 2017; Bovi *et al.*, 2018b).

After estimating the rates of respiratory water loss (WL_R) and carbon loss (CL_R) in $\text{mg kg}^{-1} \text{s}^{-1}$ based on their stoichiometric proportions and carbon dioxide production rate (R_{CO_2}), the respiratory mass loss (ML_R) is calculated from **Eq. 7**. The percentage proportions of WL_R , CL_R and ML_R with respect to the total mass loss of the fruit (ML_{tot}) were then calculated. The ML_{tot} ($\text{mg kg}^{-1} \text{s}^{-1}$) is an equivalent of TR_m and will be elaborated in **Section 3.1**.

$$ML_R = WL_R + CL_R \quad (5.7)$$

The other by-product of the respiration process, the heat, contributes to the sensible heat increase of the fruit temperature and VPD between the fruit surface and the environment. This process causes moisture loss to the surrounding atmosphere (Kang and Lee, 1998; Song *et al.*, 2002). The rate of water loss resulting from respiratory heat dissipation is calculated from **Eq. 8**.

$$WL_Q = \frac{\left(Q - C_p \frac{\partial T_s}{\partial t}\right)}{\lambda} \times 10^3 \quad (5.8)$$

$$Q = -\alpha (q_{CO_2} R_{CO_2}) \quad (5.9)$$

Where WL_Q is the rate of water vapourisation ($\text{mg kg}^{-1} \text{s}^{-1}$) owing to the respiratory heat, C_p is the specific heat of the pomegranate fruit (cv. Wonderful). A C_p value of $3302.972 \text{ J kg}^{-1} \text{ }^\circ\text{C}^{-1}$ from a previous study by Mukama *et al.*, (2018) was used in **Eq. 8**. Parameter λ is the latent heat of vaporisation of water (kJ kg^{-1}) and T_s is the surface temperature ($^\circ\text{C}$) of the fruit. Based on results from other fruit like strawberries (Bovi *et al.*, 2018b), the increase in fruit surface temperature resulting from the respiratory heat was estimated to be 0.01 , 0.07 and $0.10 \text{ }^\circ\text{C}$ at 5 , 15 and $25 \text{ }^\circ\text{C}$, respectively. The rate of respiratory heat dissipation Q ($\text{kJ kg}^{-1} \text{h}^{-1}$) is calculated from **Eq. 9**, where q_{CO_2} is the respiratory heat dissipated per kilogram CO_2 produced (GJ kg^{-1}). From the stoichiometry of oxidation of glucose and fructose, q_{CO_2} has a value of 0.011 GJ kg^{-1} . The parameter α is the conversion factor of respiration energy dissipated as heat and is in the range of 0.8 to 1.0 (Burton, 1982; Powrie and Skura, 1991; Song *et al.*, 2002). Mahajan *et al.* (2016) used a conversion factor of 0.86 to account for the effect of respiratory heat generation on transpiration model. In this current study $\alpha = 0.89$ was used as the average.

5.2.4.6 Net transpiration rate

We calculated net transpiration rate ($TR_{m\ net}$) from **Eq. 10** where CL_R ($\text{mg kg}^{-1} \text{ s}^{-1}$) is respiratory carbon loss.

$$TR_{m\ net} = (ML_{tot} - CL_R) \quad (5.10)$$

5.2.5 Statistics

Analysis of variance (ANOVA) and Pareto analysis was carried out at 95 % confidence interval using Statistica software (Statistica 13.3, Statsoft, USA) to assess the effects of temperature, RH and time, and their interaction on the mass loss, transpiration rate and respiration rate of pomegranate fruit. We separated means using Duncan's multiple range test.

5.3 Results and discussion

5.3.1 Mass loss and transpiration rate

The analysis of variance (**Table 5.1**) showed that all the storage factors (temperature, time and relative humidity), as well as their interactions, significantly influenced mass loss. **Figure 5.3a** compares the effect of storage temperature at 93 % RH because this RH is in the range of the normally recommended condition for pomegranate (Arendse *et al.*, 2014). On average, increasing storage temperature from 5 to 15 °C increased mass loss 3.7, 1.8 and 1.8 folds for fruit stored at 93, 82 and 77 % RH, respectively. This scenario characterises the importance of maintaining a cold chain during postharvest handling of fruit. **Figure 5.3b** presents the effect of RH at 25 °C, over time (depicting shelf life conditions). An increase from 77 to 82 % RH minimises mass loss 1.05, 1.10 and 1.15 folds for fruit stored at 5, 15 and 25 °C, respectively. A further increase from 82 to 93 % RH minimised mass loss 1.79 folds more, at 25 °C. This scenario supports the potential of small increments in RH to minimise mass loss during open market displays.

Transpiration rate per unit surface area (TR_A) and per unit mass (TR_m) of the pomegranate fruit were in the range of 0.036 to 0.886 $\text{mg m}^{-2} \text{ s}^{-1}$ (0.002 to 0.060 $\text{mg kg}^{-1} \text{ s}^{-1}$), respectively. Caleb *et al.* (2013) reported TR_m in the range of 0.013 – 0.194 $\text{mg kg}^{-1} \text{ s}^{-1}$ for pomegranate (cv. Acco) arils tested at 5, 10, 15 °C and 76, 86, 96 % RH. The high values in their study are probably due to the absence of a protective fruit peel and high surface area of

the arils compared to the whole fruit. Quite similar results to our findings have been reported in other studies on various fruit. Xanthopoulos *et al.* (2017) reported TR_A and TR_m in the range of 0.083 to 0.786 $\text{mg m}^{-2} \text{s}^{-1}$ and 0.007 to 0.072 $\text{mg kg}^{-1} \text{s}^{-1}$, respectively for pears tested at 0, 10, 20 °C and 70, 80, 95 % RH. Pereira *et al.* (2018) observed TR_m in the range of 0.029 – 0.056 $\text{mg kg}^{-1} \text{s}^{-1}$ for table grapes (cv. Crimson Seedless) tested at different temperatures (2.3 – 16.4 °C) and relative humidity (between 43.8 – 66.2 %) ranges. The slight differences in results from the above studies can be attributed to variances in storage conditions, product physiology and physical attributes such as surface area.

Table 5.1 Analysis of variance (ANOVA) of fruit mass loss with the experimental factors of storage temperature, time and relative humidity

Source	df	F-ratio	P-value*
Main effects			
A: temperature	2	3731.61	0.000
B: relative humidity	3	4120.08	0.000
C: time	9	2484.72	0.000
Interactions			
A × B	6	613.57	0.000
A × C	18	218.71	0.000
B × C	27	224.41	0.000
A × B × C	54	24.36	0.000
Error	527		
Total	646		

* Significant at $P \leq 0.05$; df = degree of freedom; All F-ratios are based on the residual mean square error.

From Pareto analysis (**Figure 5.4**), both temperature and RH, as well as their interaction, had a significant influence on the transpiration rate ($P < 0.05$). This is expected based on the theory where transpiration rate is VPD dependent. RH had a greater influence on TR_m compared to temperature in the range of RH and temperature tested. Similar results have been reported by Caleb *et al.* (2013) on pomegranate arils, grape tomatoes (Xanthopoulos *et al.*, 2014) and strawberries (Sousa-Gallagher *et al.*, 2013). Transpiration rate increased with increasing temperature and with decreasing RH at different gradients as depicted by the surface plot (**Figure 5.5**). Averagely, a unit increase in RH results into 4.74, 4.48 and 4.09 % decrease

in transpiration rate at 5, 15 and 25 °C, while a unit decrease in temperature resulted into 3.80, 3.51 and 5.82 % decrease in transpiration rate at 77, 82 and 93 % RH, respectively.

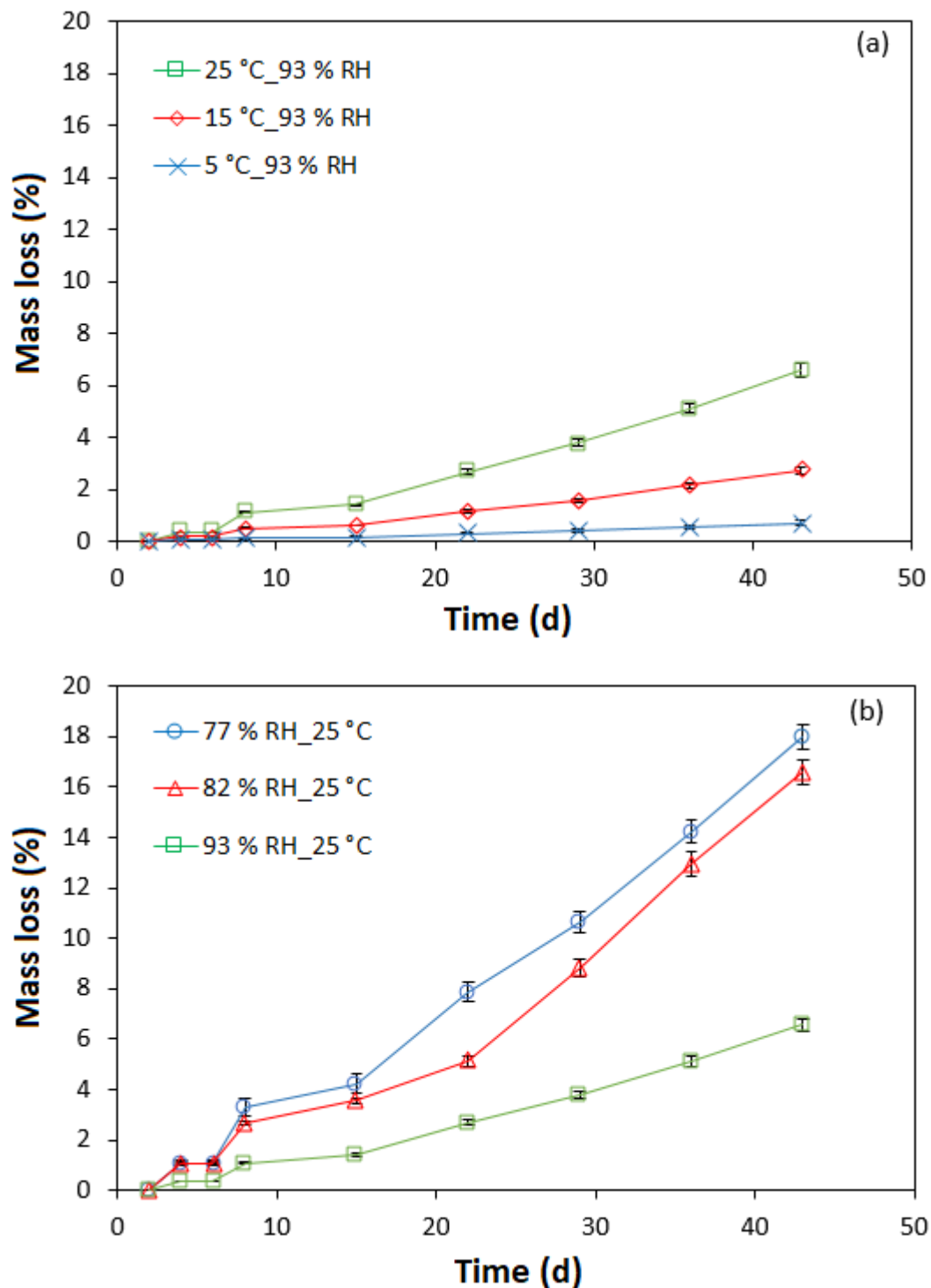


Figure 5.3 Mass loss profile of pomegranate fruit during storage. (a) shows the effect of temperature and (b) relative humidity (RH). Each point on the line graphs is the average of $n =$ six samples with vertical lines representing the estimated standard errors of the means.

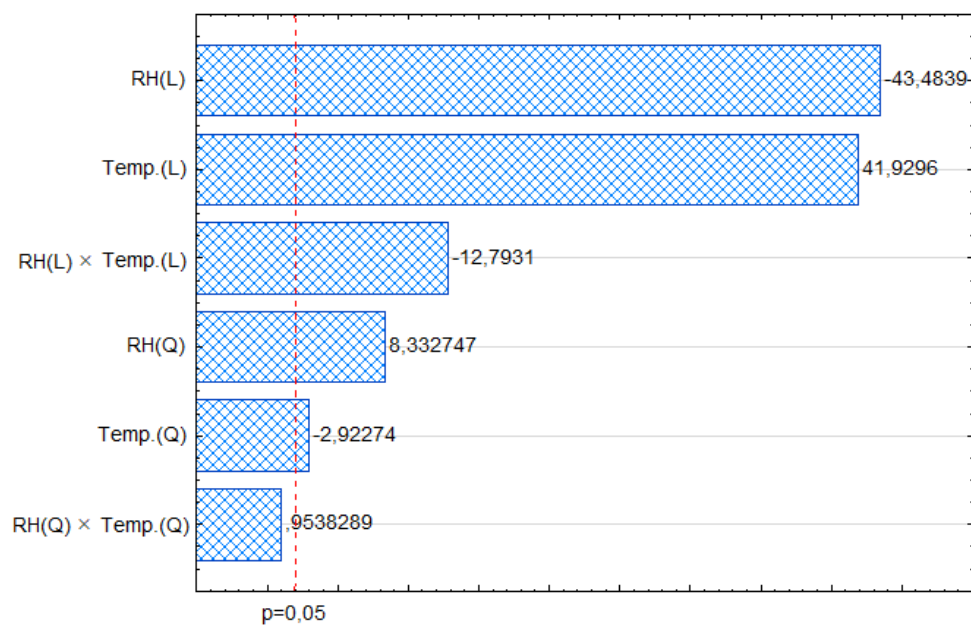


Figure 5.4 Pareto chart showing the linear (L) and quadratic (Q) effects of relative humidity (RH), storage temperature (Temp.) and their interaction on the transpiration rate of pomegranate, at $P = 0.05$ indicated with a vertical line (dashed).

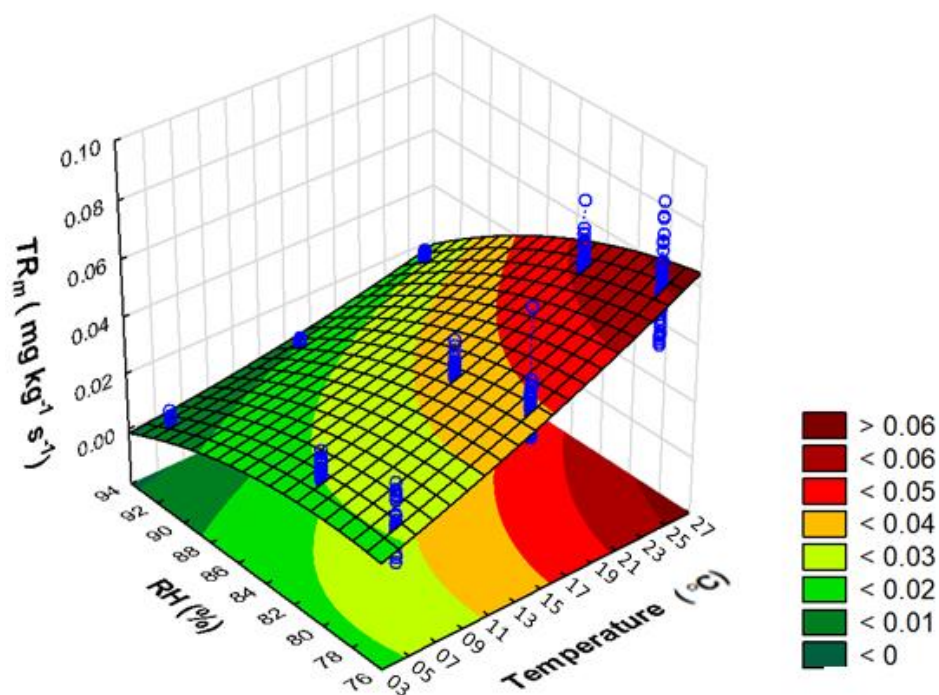


Figure 5.5 Surface plot showing the effect of storage temperature and relative humidity (RH) on the transpiration rate (TR_m) of pomegranate fruit.

Furthermore, transpiration rate (both TR_A and TR_m) positively and linearly correlated well with VPD to achieve regression coefficient (R^2) of 0.92, 0.96 and 0.99 for fruit stored at 5, 15 and 25 °C, respectively. The relationship between TR_A and VPD is presented in **Figure 5.6**. VPD ranged from 0.010 to 0.544 kPa at 5 °C/93 % RH and 25 °C/77 % RH, respectively. At VPD = 0 kPa, a mass loss rate of $TR_A = 0.029$, 0.116 and 0.274 $\text{mg m}^{-2} \text{s}^{-1}$ ($TR_m = 0.002$, 0.008 and 0.018 $\text{mg kg}^{-1} \text{s}^{-1}$) is still observed. This suggests the presence of other mass flow components such as respiration, other than transpiration water loss that contribute to the total mass loss especially at conditions of very high RH and low temperatures. Similar studies carried out on strawberries have reported the inability of temperature and RH or VPD to comprehensively account for overall mass loss (Sousa-Gallagher *et al.*, 2013; Bovi *et al.*, 2018b). Other alternative processes such as respiration have been suggested to account for mass loss especially at near saturation conditions (Mahajan *et al.*, 2016; Xanthopoulos *et al.*, 2017).

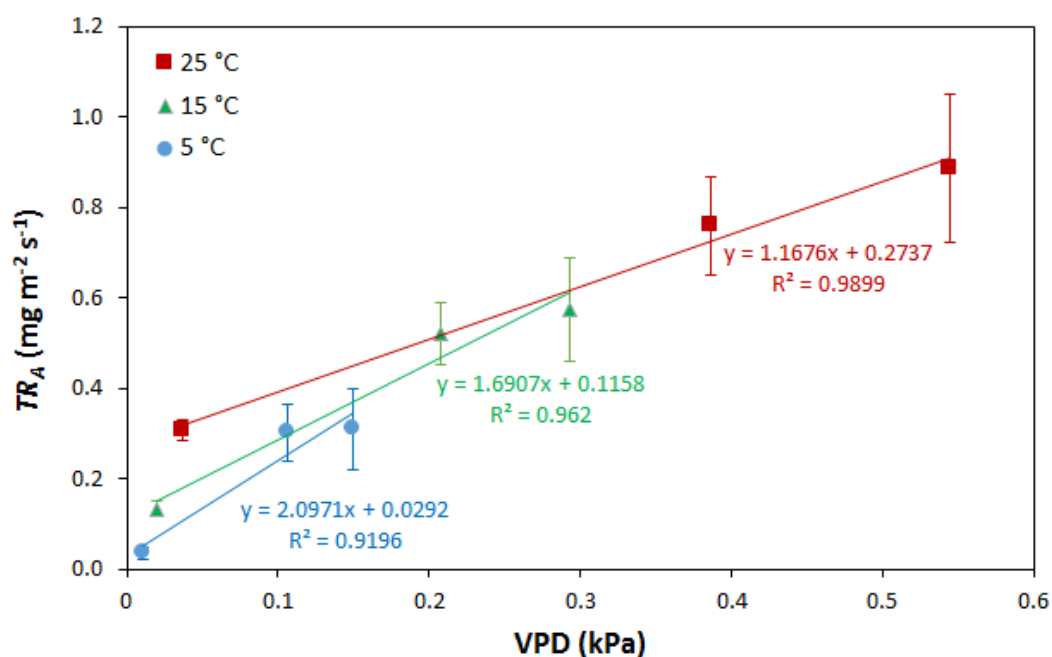


Figure 5.6 Relationship between transpiration rate (TR_A) and vapour pressure deficit (VPD) for all the tested conditions. Each point is the average of $n =$ six samples over the whole storage period, with vertical lines representing standard deviations of the means.

At saturated conditions of 100 % RH (VPD = 0 kPa), the observed residual transpiration rate of 0.002, 0.019 and 0.048 $\text{mg kg}^{-1} \text{s}^{-1}$ in strawberries, was attributed to the 0.01, 0.07 and 0.12 °C increase in surface temperature at 4, 12 and 20 °C, respectively, as a result of respiratory heat dissipation (Bovi *et al.*, 2018b). Furthermore, the authors noted that what has been in a long time modelled as transpiration rate is actually total mass loss rate (ML_{tot}) involving other

contributing mass loss components such as loss of organic volatile compounds and ethylene that are usually considered negligible. As a result, TR_m was referred to as ML_{tot} in the remaining part of the study. The contribution of net transpiration ($TR_{m\ net}$) and respiration rates to ML_{tot} of pomegranate fruit is discussed in the following sections.

5.3.2 Respiration rate

The analysis of variance among the experimental effects (**Table 5.2**) showed that the storage conditions of temperature, RH and time significantly affected the respiration rate of pomegranate fruit (cv. Wonderful) for $P \leq 0.05$. The interaction effects of temperature-time and temperature-RH were also significant. From the Pareto analysis (results not shown) temperature had the greatest linear influence on RR followed by storage duration and RH, respectively. Generally, RR varied over time, increasing for the first 13 d (**Figure 5.7**) due to stress physiological responses caused by the different storage conditions, followed by a levelling off resulting from acclimatisation until the end of the storage period. A quite similar trend is observed in pomegranates (cv. Wonderful) stored for 112 d at 5 °C and an additional 4 d at 20 °C (Atukuri *et al.*, 2017).

Table 5.2 Analysis of variance (ANOVA) of fruit respiration rate with the experimental factors of storage temperature, time and relative humidity

Source	df	F-ratio	P-value*
<i>Main effects</i>			
A: temperature	2	476.724	0.000
B: relative humidity	2	33.066	0.000
C: time	6	13.512	0.000
<i>Interactions</i>			
A × B	4	5.704	0.000
A × C	12	1.863	0.046
B × C	12	1.245	0.261
A × B × C	24	1.042	0.421
Error	119		
Total	181		

* Significant at $P \leq 0.05$; df = degree of freedom; All F-ratios are based on the residual mean square error.

The effect of temperature on RR is presented in **Figure 5.7a** for fruit stored at 93 % RH. As pomegranate is a non-climacteric fruit, these are the expected results. At 13 d of storage, RR decreased from 12.59 $\mu\text{g kg}^{-1} \text{s}^{-1}$ at 25 °C to 8.67 and 1.92 $\mu\text{g kg}^{-1} \text{s}^{-1}$ at 15 °C and

5 °C, respectively. Reducing storage temperature from 15 to 5 °C decreased RR by 78.8, 70.6 and 70 % at 93, 82, and 77 % RH, respectively. Caleb *et al.* (2012a) reported a 67 and 70 % decrease in RR of pomegranate (cv. Acco and Herskawitz) whole fruit and arils, respectively, for a similar temperature reduction. Comparable to our results, the authors reported RR as high as $9.49 \mu\text{g kg}^{-1} \text{s}^{-1}$ for whole fruit pomegranate stored at 15 °C. The variation of RR with RH is demonstrated in **Figure 5.7b**. For RR at 5 °C and three levels of RH, a high RH resulted in a low RR while a higher RR was observed at the rest of the RH conditions. The higher RR at lower RH could be attributed to elevated physiological stress resulting from increased VPD and higher moisture loss.

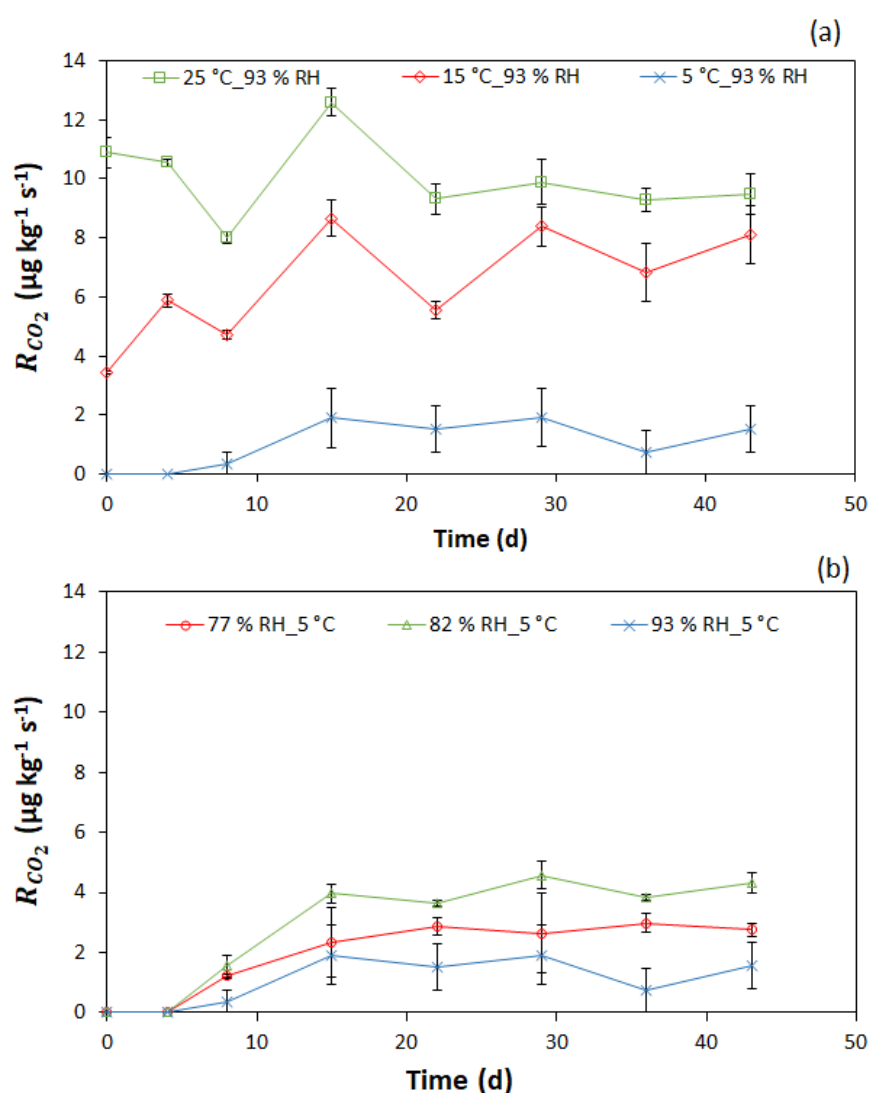


Figure 5.7 Rate of carbon dioxide production (R_{CO_2}) profile for pomegranate fruit during storage. (a) shows the effect of storage temperature and (b) relative humidity (RH). Each point on the line graphs is the average of $n =$ six samples with vertical lines representing the estimated standard errors of the means.

5.3.3 Water activity

The water activity of the different fruit parts did not differ among storage temperature-RH combinations; therefore, the average values were presented in **Table 5.3**. Water activity decreased from the inside to the outside of the fruit, indicating a water vapour gradient. The average a_w at the centre (arils) and the outer most part (exocarp) of the fruit was 0.964 and 0.942, respectively. This implies that the direction of water movement is from the inside of the fruit to the surrounding. A relatively higher water activity of 0.984 ± 0.01 was reported for pomegranate arils (cv. Acco) stored under different temperatures (5, 10 and 15 °C) and RH (76, 86 and 96 %) conditions (Caleb *et al.*, 2013). However, Xanthopoulos *et al.* (2017) observed a relatively lower average value of 0.924 ± 0.05 for pears stored at 20 °C and 80 % RH.

Table 5.3 Water activity (a_w) of pomegranate fruit fractions. The values are means (\pm standard error of the mean) of all the measurement taken at 5, 15 and 25 °C after 43 d of fruit storage at the same temperature

Fruit fraction	a_w
Arils	0.9643 ± 0.0036
White membrane	0.9609 ± 0.0042
Mesocarp	0.9522 ± 0.0057
Exocarp	0.9418 ± 0.0093
Overall average	0.9548 ± 0.0032

5.3.4 The contribution of respiration and transpiration to overall mass loss

5.3.4.1 Net transpiration rate

The net transpiration rate $TR_{m\ net}$ gives a clear indication of the amount of water loss, while the respiratory carbon loss (CL_R) corresponds to the theoretical dry matter loss. Calculations have been carried out based on the assumption that the main oxidation substrate is glucose and fructose. The $TR_{m\ net}$ ranged from 0.002 to 0.055 mg kg⁻¹ s⁻¹ at 5 °C/93 % RH and 25 °C/77 % RH, attributed to differences in VPD of 0.010 and 0.544 kPa, respectively (**Figure 5.8a-c**). As expected, the biggest contribution to ML_{tot} lies in water vapour lost compared to substrate carbon loss (Bovi *et al.*, 2018b). The contribution of $TR_{m\ net}$ to ML_{tot} was higher at lower RH compared to higher RH values, irrespective of the storage temperature (**Figure 5.8d-f**). For

example, at 5 °C, $TR_{m\ net}$ contributed 97.2, 95.8 and 77.8 % at 77, 82 and 93 % RH, respectively. Furthermore, $TR_{m\ net}$ contribution increased with decreasing storage temperature, especially at 77 and 82 % RH.

5.3.4.2 Respiratory mass components

The rate of mass loss in the respiratory components and their contribution to the total mass loss are presented in **Figures 5.8a-c** and **5.8d-f**, respectively. In this case the contribution of the respiratory mass loss (ML_R) to ML_{tot} was the exact opposite of the contribution of $TR_{m\ net}$. It is important to note that though the water loss component WL_R is accounted for in the $TR_{m\ net}$, it is sourced from the respiration process. ML_R ranged between 7 % (at 5 °C/77 % RH) to > 50 % (at 5 °C/93 % RH and 15 °C/93 % RH). ML_R increased with increasing RH irrespective of the storage temperature. Increasing the RH from 77 to 82 to 93 % at 5 °C increased ML_R from 7.2 to 10.4 to 57.5 %, respectively. On the other hand, increasing the temperature from 5 to 15 to 25 °C at 77 % RH, increased ML_R from 7.2 to 12.7 to 16.9 %, respectively. The effect of temperature was more elaborate at lower RH of 77 and 82 % than at 93 %. The increase of ML_R was greater with increasing RH than with temperature probably because the effect of RH was more quadratic in function while the influence of temperature was more linear according to the Pareto analysis (results not shown). Despite the generally low mass loss rates at conditions of low temperature-high RH, weight loss has a cumulative impact and therefore the contributions of ML_R become significant during prolonged storage of large volumes of fruit. Thus minimising ML_R can have a huge financial impact on the global export market, given that pomegranates are considered as luxurious fruit that sell well in the higher market segment (CBI, 2019).

The biggest source of ML_R is the WL_R as compared to CL_R , irrespective of the storage conditions. The contribution of WL_R and CL_R ranged from 0.001 to 0.006 mg kg⁻¹ s⁻¹ and 0.001 to 0.004 mg kg⁻¹ s⁻¹, respectively, with lowest rates at 5 °C/77 % RH and highest rates at 25 °C, 77 and 82 % RH (**Figure 5.8a-c**). The contributions of WL_R and CL_R to ML_{tot} can go as high as 35 and 23 % at high RH 93 % (VPD = 0.010-0.037 kPa) and as low as 4 and 3 % at lower RH of 77 % (VPD = 0.150-0.544 kPa) (**Figure 5.8d-f**). In our current study on pomegranate (non-climacteric) fruit, WL_R averaged at 13.3, 18.5 and 15 % at 5, 15 and 25 °C, respectively, in the range of 77-93 % RH (VPD = 0.010 and 0.544 kPa). In a similar study on climacteric fruit, Xanthopoulos *et al.* (2017) reported a comparably similar respiratory water loss of 39 %

for pears stored at 20 °C and 95 % RH (VPD ~ 0.0 kPa) with an average of 8, 14 and 23 % at 0, 10 and 20 °C, respectively, in the range of 70-95 % RH (VPD = 0.0-0.522 kPa).

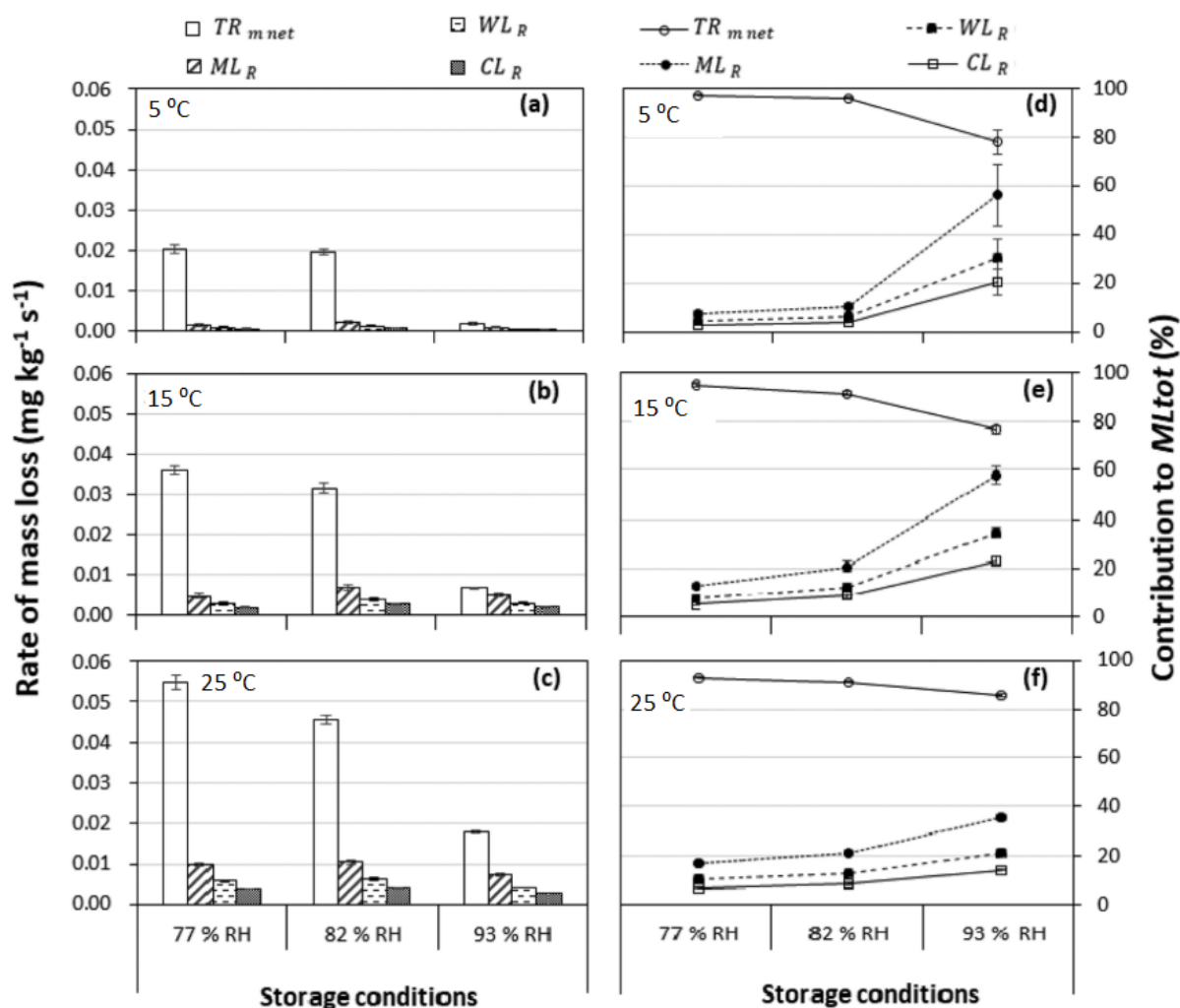


Figure 5.8 Mass loss components of pomegranate fruit (cv. Wonderful) stored at different temperature-relative humidity (RH) combinations. The rate of mass loss of the different components (a-c) and their percentage contribution to the total mass loss of the fruit (d-f). $TR_{m\ net}$, net transpiration rate; WL_R , respiratory water loss CL_R , respiratory carbon loss; ML_R , respiratory mass loss; ML_{tot} , total mass loss. The vertical lines represent the estimated standard error of the mean for $n = 21$ (three replicates \times seven sampling times).

From stoichiometry, the more substrate consumed, the higher the carbon loss during respiration. The percentage contribution of CL_R to ML_{tot} in the range of 3 to 23 % in this study is comparable to the contribution of substrate consumption of 3 to 20 % in non-climacteric fruit, for strawberries stored under similar conditions of 4, 12, 20 °C and 76, 86 and 96 % RH (VPD ~ 0.0-0.575 kPa) (Bovi *et al.*, 2018b). Particularly for strawberries stored at 76 % RH (VPD ~ 0.190-0.575 kPa), the rate of respiratory mass loss calculated with respect to substrate

consumption accounted for 3, 5 and 8 % of the ML_{tot} at 4, 12 and 20 °C, respectively (Bovi *et al.*, 2018b). Similarly, in the current study, the rate of carbon loss (CL_R) accounted for 3, 5 and 8 % of ML_{tot} at 5, 15 and 25 °C, respectively, for pomegranate fruit stored at 77 % RH (VPD = 0.150-0.544 kPa).

5.3.4.3 The potential of dissipated respiratory heat

It has been established that besides respiratory carbon (substrate) loss, respiratory heat plays an important role in the mass loss of fresh produce especially in high humidity conditions such as inside packages (Saltveit, 2002; Bovi *et al.*, 2018a). **Figure 5.9a-b** shows the rate of respiratory heat dissipation and the potential mass loss owing to respiratory heat. The respiratory heat (Q) and the resulting water loss (WL_Q) increases with increasing temperature. This is because of high heat dissipation, due to increased energy demands of the fruit at high storage temperatures. The estimated values of Q ranged between 0.017 and 0.129 J kg⁻¹ s⁻¹ causing a water loss rate (WL_Q) of 0.001 and 0.020 mg kg⁻¹ s⁻¹, respectively. Mahajan *et al.* (2016) attributed the continued loss of moisture from strawberries, tomatoes and mushrooms to the generated respiratory heat, during storage at saturated humidity conditions. Respiratory heat increases the surface temperature of the product, thereby increasing the VPD between the product and the surrounding environment resulting into an increased mass loss (Sastry and Buffington, 1983; Kang and Lee, 1998; Bovi *et al.*, 2018b). However, at high transpiration rates due to conditions of high VPD, the effect of respiratory heat could be counteracted with evaporative cooling at the surface of the product causing vapour pressure lowering (Sastry and Buffington, 1983). Future studies should carefully consider direct ways of quantifying real-time respiratory heat of produce and surface temperature determination.

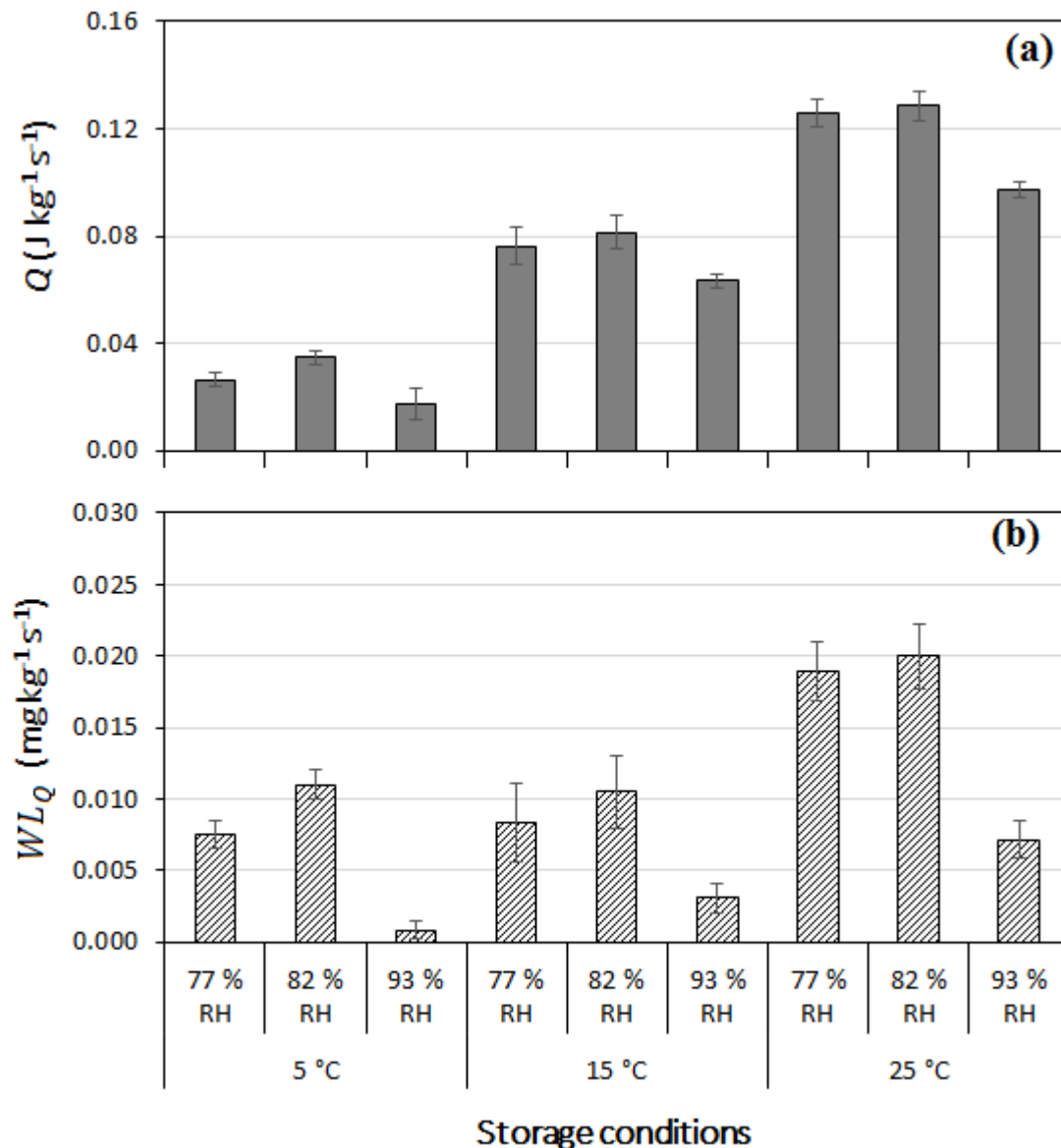


Figure 5.9 The rate of respiratory heat dissipation Q (a) and the potential rate of respiratory water loss WL_Q (b) for pomegranate fruit (cv. Wonderful) stored at different temperature-relative humidity (RH) conditions. The vertical lines represent standard error of the mean for $n = 21$ (three replicates \times seven sampling times).

5.4 Conclusions

A key finding from this study is that respiration rate contributes to the total mass loss rate of fresh pomegranate fruit. Most of the mass loss occurring in fresh pomegranate fruit was explained by the VPD of 0.01-0.54 kPa (in the range of the tested conditions) between the fruit surface and the surrounding environment. However, the observed mass loss rate of $TR_A = 0.029\text{-}0.274 \text{ mg m}^{-2} \text{ s}^{-1}$ ($TR_m = 0.002\text{-}0.018 \text{ mg kg}^{-1} \text{ s}^{-1}$) at VPD = 0 kPa suggests the presence of other mass flow components such as respiration, other than transpiration. Water loss and

carbon losses due to the respiratory process contributed up to 35 % and 23 % at high RH (93 %) and 4 % and 3 % at lower RH (77 %) of the total mass loss, respectively, and the rest was due to VPD. The specific contribution of these respiratory mass flow components to transpiration and total mass loss are important to consider in developing predictive models and strategic control techniques, especially at high relative humidity conditions. Furthermore, this study gives insight and promotes the quantification of fresh produce water loss with more precision. Storage conditions of temperature, relative humidity and time had a significant influence on the mass loss processes and therefore important to consider in predictive model development.

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CHAPTER 6

DETERMINING WATER TRANSPORT PROPERTIES (DIFFUSIVITY AND PERMEABILITY) OF POMEGRANATE FRUIT TISSUES

With regard to Chapter 6, pages 150–167, the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Research, data collection and analysis, and writing of chapter	80

The following co-authors have contributed to Chapter 6, pages 150–167:

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Declaration with signature in possession of candidate and supervisor Signature of candidate	26/02/2020 Date
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Declaration by co-authors

The undersigned hereby confirm that:

1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 6, pages 150–167,
2. no other authors contributed to Chapter 6, pages 150–167 besides those specified above, and
3. potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 6, pages 150–167 of this dissertation.

Signature	Institutional affiliation	Date
Declaration with signature in possession of candidate and supervisor	Department of Horticultural Sciences, Stellenbosch University	26/02/2020
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Chapter 6

6 Determining water transport properties (diffusivity and permeability) of pomegranate fruit tissues

Abstract

Excessive water loss is one of the most important factors influencing the postharvest quality of fresh fruit along the value chain from harvest to consumption, ultimately resulting in huge financial losses. Water transport properties characterise the rate and the resistance of water movement across product tissues and membranes. For the first time, water transport properties of the non-edible pomegranate fruit tissues (exocarp, mesocarp and white membrane) were determined under cold and shelf storage conditions. A diffusion cup experimental setup was used to monitor moisture flux across the tissues and then moisture diffusivity (D) and permeability (K) were calculated based on Fick's law. The effect of tissue location on the fruit and determination temperature were also investigated. Across all tested conditions, D and K were lower in the exocarp tissues than in the mesocarp tissues of the peel. For example, a D of $1.80\text{--}1.96 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$ and K of $1.31\text{--}1.43 \times 10^{-11} \text{ kg m}^{-1} \text{ pa}^{-1} \text{ s}^{-1}$ were observed in the exocarp tissues at 23 °C before storage compared to $18.53\text{--}22.45 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$ and $13.51\text{--}16.37 \times 10^{-11} \text{ kg m}^{-1} \text{ pa}^{-1} \text{ s}^{-1}$ in the mesocarp tissues, respectively. Very low moisture diffusivity and permeability were observed in the white membrane as compared to the exocarp and mesocarp tissues. These findings suggest the white membrane as a very protective tissue (highest resistance) against moisture loss from the edible portion (arils) of the fruit.

6.1 Introduction

Excessive water loss is one of the most important factors influencing the postharvest quality of fresh fruit along the value chain from harvest to consumption, ultimately resulting in huge financial losses. A weight loss of 5 % is sufficient to cause shrivelling in pomegranate fruit (Elyatem and Kader, 1984; Lufu, 2017). Even in the cases of no visible shrivelling, water loss can undesirably affect the visual appearance, flavour and textural properties of the fruit (Pareek *et al.*, 2015). Excessive water loss results in browning of the peel and arils, hardening of the rind (Kader *et al.*, 1984; Artés *et al.*, 2000; Caleb *et al.*, 2012). Water loss is influenced by both external environmental factors such as temperature, relative humidity, airflow rate and inherent

product factors such as surface area, presence of waxy cuticle, surface stomata, lenticels and micro-cracks.

Water movement from within the fruit across various tissues to the surface where it is ultimately lost to the surrounding occurs by convection, molecular diffusion and capillary diffusion mechanisms depending on the driving force responsible for the movement (Datta and Zhang, 1999; Nguyen *et al.*, 2006). Convective mass transfer, molecular diffusion and capillary diffusion mechanisms are driven by a pressure gradient, concentration gradient and the relative interaction of cohesive and adhesive forces between the liquid and solid phase, respectively (Datta and Zhang, 1999; Nguyen *et al.*, 2006).

Water transport properties characterise the rate and the resistance of water movement across product tissues and membranes. These properties are very important in modelling and simulation of fruit storage, processing and preservation processes, designing of efficient packaging such modified atmosphere packaging (MAP) bags and appropriate formulation and implementation surface waxing and coating. The most common water transport properties applied on fruit and fruit tissues include water flux, water permeability or conductivity, hydraulic permeability, and moisture diffusivity. Water flux describes the rate at which water moves across a material per unit cross-sectional area. Permeability on the other side describes the ease with which water moves across a given material. Water permeance/permeability has been calculated on whole intact fruit such as in ‘Braeburn’ apples (Maguire *et al.*, 1999), ‘Bartlett’, ‘Beurre Bosc’, ‘Doyenne du Comice’ and ‘Packham’s Triumph’ pears (Amarante *et al.*, 2001), Japanese plum cultivars ‘African Rose’, ‘Angeleno’, ‘Ruby Sun’, ‘Fortune’ and ‘Ruby Star’ (Kritzinger *et al.*, 2018), ‘Laetitia’ and ‘Songold’ plums (Kritzinger and Lötze, 2019). However, in such cases, details on the water movement within the fruit are lost through generalisation and yet fruit are highly heterogeneous and complex in structure. Therefore, the determination of water transport properties of the different tissues within the fruit is very important in facilitating the detailed investigation of the spatial-temporal moisture distribution within the fruit. As a result, water diffusivity of the cuticle, cutin, wax and flesh tissues have been specifically investigated for. ‘Jonagold’, ‘Elstar’ and ‘Jonagored’ apples (Veraverbeke *et al.*, 2003b) and ‘conference’ pears (Nguyen *et al.*, 2006). Results from these previous studies showed that water transport properties are greatly influenced by biological variability and therefore varies among fruit types, cultivars and within different fruit tissues.

Pomegranate fruit has a complex structure compared to apples and pears, consisting of the non-edible thick tough leathery rind and the arils as the edible portion. The rind consists of the outer tough peel (the exocarp or flavedo) and the inner spongy peel (the mesocarp or albedo) which surrounds the arils. The mesocarp is lined by an inner epithelium (the inner epidermal layer or white divider membrane). Each aril consists of a single seed surrounded by a juicy sac. The arils are attached to the projections of the inner walls of the mesocarp and are clustered in segments which are separated by the single-cell layers, the inner epidermal membranes. The different tissues within the pomegranate fruit are expected to have different water transport properties and therefore approximating the fruit to a homogenous solid or extrapolation from other previously studied fruit types is considered unrealistic. On the other hand, literature on the water transport properties of pomegranate tissues is lacking. Therefore, the current study aimed to determine the water transport properties of pomegranate fruit tissues. The study was carried out on 'Wonderful' cultivar, which is the most importantly grown and exported in South Africa. The specific focus of the study was on the experimental measurement of the transport properties at different temperatures. Furthermore, the effect of location on the fruit as well as fruit storage were investigated.

6.2 Materials and methods

6.2.1 Fruit acquisition

Pomegranate fruit (*Punica granatum* L.) of cultivar 'Wonderful' were harvested at commercial maturity from a commercial orchard situated in Porterville, Wellington (33° 38' S, 19° 00' E), Western Cape Province, South Africa. Fruit were transported in ventilated plastic trays cushioned with paper pads to the postharvest research laboratory, Stellenbosch University. Sorting of fruit was carried out to ensure size uniformity and that the fruit were free from surface defects such as cracks. Fruit were packed in dozens inside single layer display type paper cartons, cushioned with paper trays at the bottom.

6.2.2 Experimental design

Two cartons of 12 fruit each were stored at 7 °C and 90 % RH for 42 d and 23 °C and 58 % RH for eight days, respectively. Twelve fruit were randomly sampled for WVTR cup experiment before storage and at the end of each storage regime. The sampled fruit were then stored in modified atmosphere packaging (MAP) plastic liners inside the cartons at 7 °C and 90 % RH to minimize moisture loss shortly before analysis. From each set of twelve sampled fruit,

WVTR was conducted separately at 23 °C/58 % RH, 15 °C/70 % RH and 7 °C/90 % RH using tissue samples from four different fruit for each condition.

6.2.3 Sample preparation

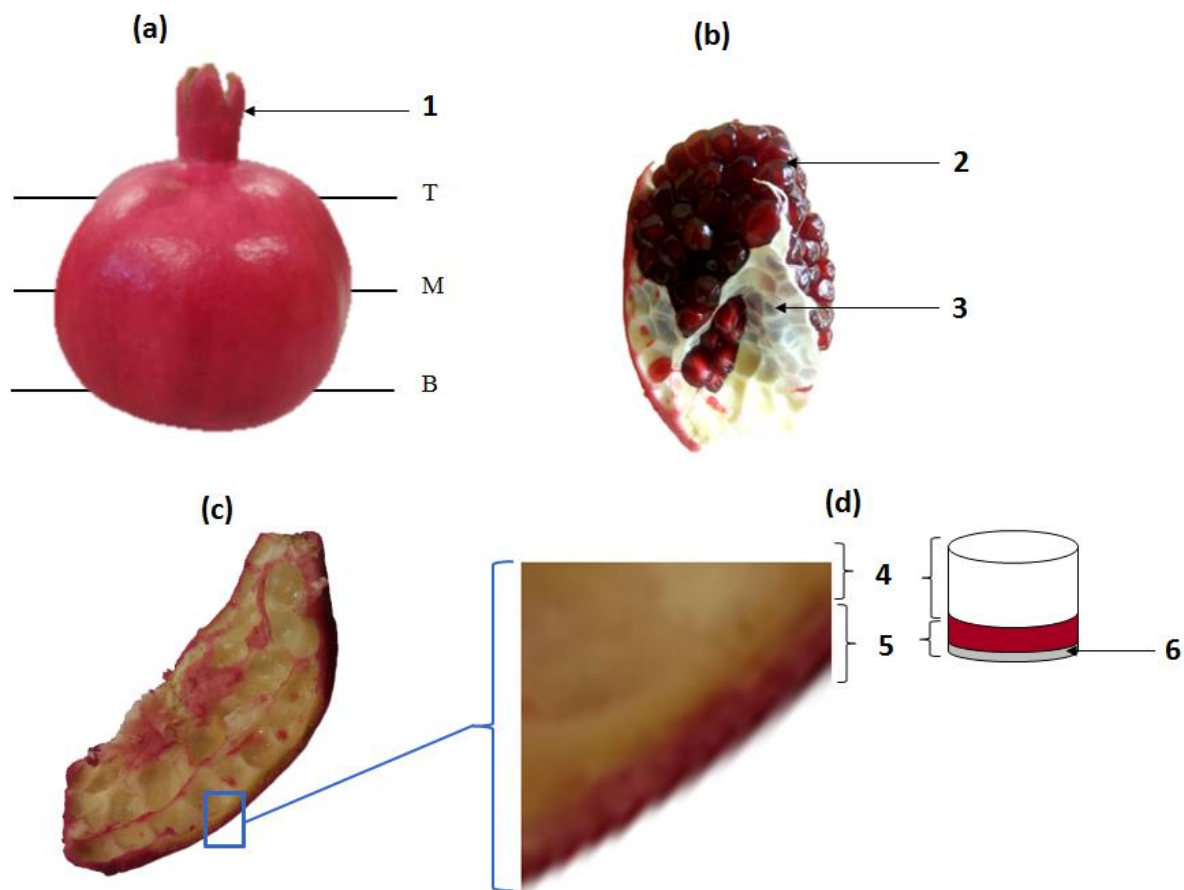


Figure 6.1. A representation of the different tissues of pomegranate fruit (cv. Wonderful). Whole fruit showing the different location of sampling (a), fruit segment showing the edible portion (b), the rind/peel (c) and the layers of the rind (d). Samples were excerpted from the top (T), mid (M) and bottom (B) locations. 1-calyx/crown, 2-arils, 3-white divider membrane (inner epidermis), 4-exocarp (outer peel), 5-mesocarp (inner peel) and 6-waxy cuticular layer.

The whole pomegranate fruit were carefully cut open to separate the non-edible from the edible portions (**Figure 6.1a-d**). The fruit were sectioned top-bottom into three to four segments (**Figure 6.1a-c**). Extra care was taken to avoid possible damage of the white divider membrane/septum (inner epidermis) from the sharp blade and tare when separating from the arils (edible portion). Discs of diameter 2.5 cm were marked on the peel surface of the fruit segments using a hollow tube of known diameter. The discs were then cut out of the top, mid and bottom locations of each segments using sharp specialised sectioning razor blades. Therefore, about

three to four discs were obtained from each location per fruit. However, about one to two tissue discs were successfully attainable from the divider membranes per fruit.

The peel discs were further sectioned to separate the exocarp (the outer peel) from the mesocarp (the inner peel) tissue fraction using the sharp sectioning blades (**Figure 6.1d**). In this case, extra care was taken to minimise rubbing and damaging of the tissue surfaces. Furthermore, sectioned samples were immediately kept in closed containers at 4 °C to minimise enzymatic browning. The discs were blotted with tissue paper to remove excess moisture. The thickness of the discs was measured using a digital Vernier calliper (Mitutoyo, Kawasaki, Japan, ± 0.01 mm) at four different and opposite points along the circumference of the disc surface. The discs were then mounted in stainless steel diffusion cell. The arils were not included in this experiment.

6.2.4 Moisture diffusion experimental set up

A modification of the dry cup technique (ASTM, 2005 method E96-95) was used to gravimetrically monitor water diffusion across the different fruit tissues (**Figure 6.2**). The diffusion cup setup consisted of the aluminium test cup (diameter 5.6 cm and depth 1.5 cm) with open top-screw lid (Comar International, Cape Town, South Africa). The cup was fitted with an O-ring and grease to provide proofing against moisture and air. The tissue sample was placed firm between aluminium hollow plates with waterproof sealant. Each plate had a hole of diameter 2 cm, thus exposing the inner and outer sides of the tissue. A screw-on hollow aluminium lid was then used to fasten the plates onto the aluminium cup filled with distilled water. The inner side of the tissues was placed in contact with water, while the outer side of the tissues was exposed to the surrounding air. The whole set up was then conditioned for two hours in an environmental test chamber (Sanyo Electric Co., Osaka, Japan) with controlled constant air movement (1 m s^{-1}), temperature and relative humidity. The loss in weight of each cup was monitored at regular intervals for 48 hours.

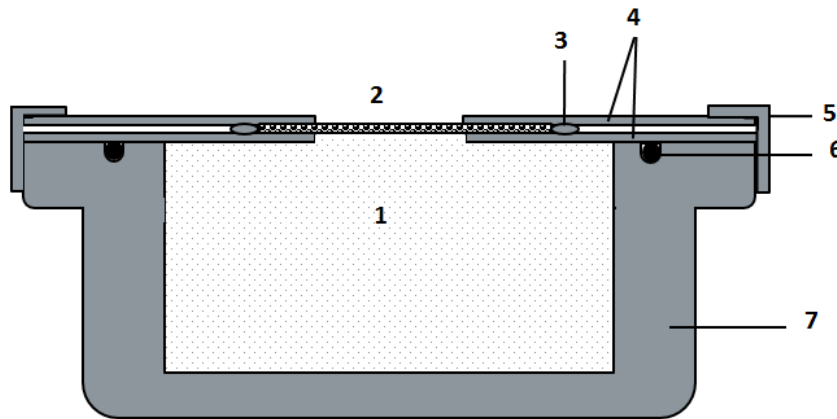


Figure 6.2. Diffusion cup experimental setup. 1-distilled water, 2-tissue sample, 3-sealant, 4-inner and outer aluminium plates, 5-cup lid, 6-rubber O-ring and 7-aluminium cup.

6.2.5 Calculation of water transport properties

Water flux across the fruit tissues was calculated using **Equation 6.1**. The water permeability or conductivity and effective diffusivity were then estimated from Fick's diffusion law using **Equation 6.2** and **Equation 6.3**, respectively, by assuming steady-state conditions and a constant linear concentration gradient within the thin tissue membranes.

$$J = \frac{M_t - M_i}{A \times t} \quad (6.1)$$

$$K = -\frac{J \Delta x}{\Delta \psi} \quad (6.2)$$

$$D_{eff} = -\frac{J \Delta x}{\Delta C} \quad (6.3)$$

Where J ($\text{kg m}^{-2} \text{s}^{-1}$) is the water flux, M_t (kg) is the mass of the diffusion cup setup at a time t (s), and M_i (kg) is the initial mass of the setup and A is the effective surface area of the tissue. Parameter K ($\text{kg m}^{-1} \text{Pa}^{-1} \text{s}^{-1}$) is water permeability or conductivity, D_{eff} ($\text{m}^2 \text{s}^{-1}$) is the effective diffusivity, Δx is tissue thickness (m), while $\Delta \psi$ (Pa) and ΔC (kg m^{-3}) represent water potential and moisture concentration gradients between of the inner and outer sides of the tissue, inside and outside the cup, respectively.

6.2.6 Statistics

Analysis of variance (ANOVA) was carried out at 95 % confidence interval using Statistica software (Statistica 13.3, Statsoft, USA) to assess the effects storage conditions, location on the fruit, tissue type, determination temperature and their interactions during the experiment.

6.3 Results and discussion

6.3.1 Analysis of variance

Table 6.1 Analysis of variance (ANOVA) of moisture permeability and diffusivity for pomegranate (cv. Wonderful) fruit tissues with respect to experimental factors of storage duration, temperature of determination, tissue type and location on the fruit

Effects	df	F-ratio	P-value*
<i>Main effects</i>			
A: Storage duration	4	4.042	0.003133
B: Temperature of determination	4	147.438	0.000000
C: Tissue type	4	313.717	0.000000
D: Location on the fruit	4	0.854	0.491662
<i>Interactions</i>			
A × B	8	0.720	0.673608
A × C	8	6.765	0.000000
B × C	8	50.381	0.000000
A × D	8	0.681	0.708030
B × D	8	0.936	0.486000
C × D	8	1.011	0.426613
A × B × C	16	2.218	0.004393
A × B × D	16	0.976	0.481846
A × C × D	16	0.724	0.770371
B × C × D	16	1.416	0.129251
A*B*C*D	32	0.786	0.794873
Error	444		

*Significant at $P \leq 0.05$; df = degree of freedom; All F-ratios are based on the residual mean square error.

Generally, the main experimental factors of storage duration, type of fruit tissue and temperature of determination had a significant influence on the water transport properties (moisture diffusivity and permeability) as shown in **Table 6.1**. The interaction between storage duration and tissue type as well as between temperature of determination and tissue type greatly and significantly influenced the moisture diffusivity and permeability. Furthermore, the combined effect of storage duration, temperature of determination and tissue type had a significant impact on the transport properties of the tissue. However, tissue location on the fruit as well as its combined influence with our effects did not significantly influence water transport properties.

6.3.2 Effect of tissue type

Moisture diffusivity (**Figure 6.3a-i** and **6.4**) and permeability (**Figure 6.5a-i** and **6.6**) varied greatly among the different fruit tissue type. Across all tested conditions, D and K were lower

in the exocarp tissues than in the mesocarp tissues of the peel. For example, a D of $1.80\text{--}1.96 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$ and K of $1.31\text{--}1.43 \times 10^{-11} \text{ kg m}^{-1} \text{ pa}^{-1} \text{ s}^{-1}$ were observed in the exocarp tissues at 23°C before storage compared to $18.53\text{--}22.45 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$ and $13.51\text{--}16.37 \times 10^{-11} \text{ kg m}^{-1} \text{ pa}^{-1} \text{ s}^{-1}$ in the mesocarp tissues, respectively. This can be attributed to the loosely packed parenchyma cells found within the mesocarp tissues as compared to the tightly packed cells in the exocarp tissues. The exocarp (outer peel) is harder, woody, tougher and more leather-like compared to the spongy mesocarp (inner peel) (Stover and Mercure, 2007). Furthermore, the exocarp of pomegranate is outlaid with a waxy cuticular layer that protects the fruit against excessive moisture loss (Zhang *et al.*, 2015). For these reasons, the exocarp offers more resistance to moisture movement as compared to the mesocarp. In a similar study, diffusion coefficient varied significantly with tissue type, however, it was dramatically higher in the inner cortex ($435.9 \times 10^{-13} \text{ m}^2 \text{ s}^{-1}$) than in the outer cortex tissues ($10.5 \times 10^{-13} \text{ m}^2 \text{ s}^{-1}$) at 20°C (Nguyen *et al.*, 2006). These results reported in pear tissues are much lower than the ones observed in this study on pomegranate tissues. A very large variability of moisture diffusivity has been reported in food products ranging from 10^{-12} to $10^{-8} \text{ m}^2 \text{ s}^{-1}$ (Zogzas *et al.*, 1996). The variability is attributed to several factors including conditions of experimental procedures, methods of data treatment, product structure, compositional and physiological properties of the product (Guo *et al.*, 1996; Zogzas and Maroulis, 1996).

Removing the surface waxy layer greatly and significantly increased the transport properties of the exocarp (**Figures 6.3b, e, f and 6.5b, e, f**). For example, the D ($1.80\text{--}1.96 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$) and K ($1.31\text{--}1.43 \times 10^{-11} \text{ kg m}^{-1} \text{ pa}^{-1} \text{ s}^{-1}$) of the exocarp tissue increased 2.8–3.2 folds to $5.17\text{--}6.24 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$ and $4.04\text{--}4.55 \times 10^{-11} \text{ kg m}^{-1} \text{ pa}^{-1} \text{ s}^{-1}$ in the exocarp with no wax layer at 23°C before storage, respectively. Quite similar to our findings, the extraction of cuticular waxes from the leaf membranes increased water permeability by a factor 300 – 500 (Schönherr, 1979). Furthermore, the authors report that water permeability of cuticles is completely determined by the waxes. These results demonstrate the significant role of the waxy layer in minimising water loss during storage. During fruit storage, water loss occurs across the epidermis with the waxy layer as the most important transport resisting (Jenks *et al.*, 1994; Veraverbeke *et al.*, 2001b). A waxy layer covers the numerous micro-cracks and clogs several lenticels or stomata on the surface of the fruit openings as observed in apple fruit (Veraverbeke *et al.*, 2001a,b, 2003a). Very low moisture diffusivity and permeability were observed in the white membrane as compared to the exocarp and mesocarp tissues. The D of $0.19 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$ and K of $0.14 \times 10^{-11} \text{ kg m}^{-1} \text{ pa}^{-1} \text{ s}^{-1}$ were reported for the white membrane tissue at 23°C

before storage. These findings suggest the white membrane as a very protective tissue (highest resistance) inside the pomegranate fruit against moisture loss. This white membrane (inner epidermis) is the tissue that directly covers the arils (edible portion). The membrane has smooth surfaces and is made up of a single layer of small-sized palisade cells (Zhang *et al.*, 2015). These findings explain why water loss in pomegranate fruit is primarily and majorly from the peel (exocarp and mesocarp) as compared to the arils. Therefore, even though pomegranate fruit are highly susceptible to moisture loss across the numerous pores of the surface of the peel, the edible portion of the fruit is largely preserved from excessive moisture loss by the inner epidermal membrane. This increases the longevity of the edible portion of the fruit making it relevant for further processing into juices and jam at a much later stage after storage.

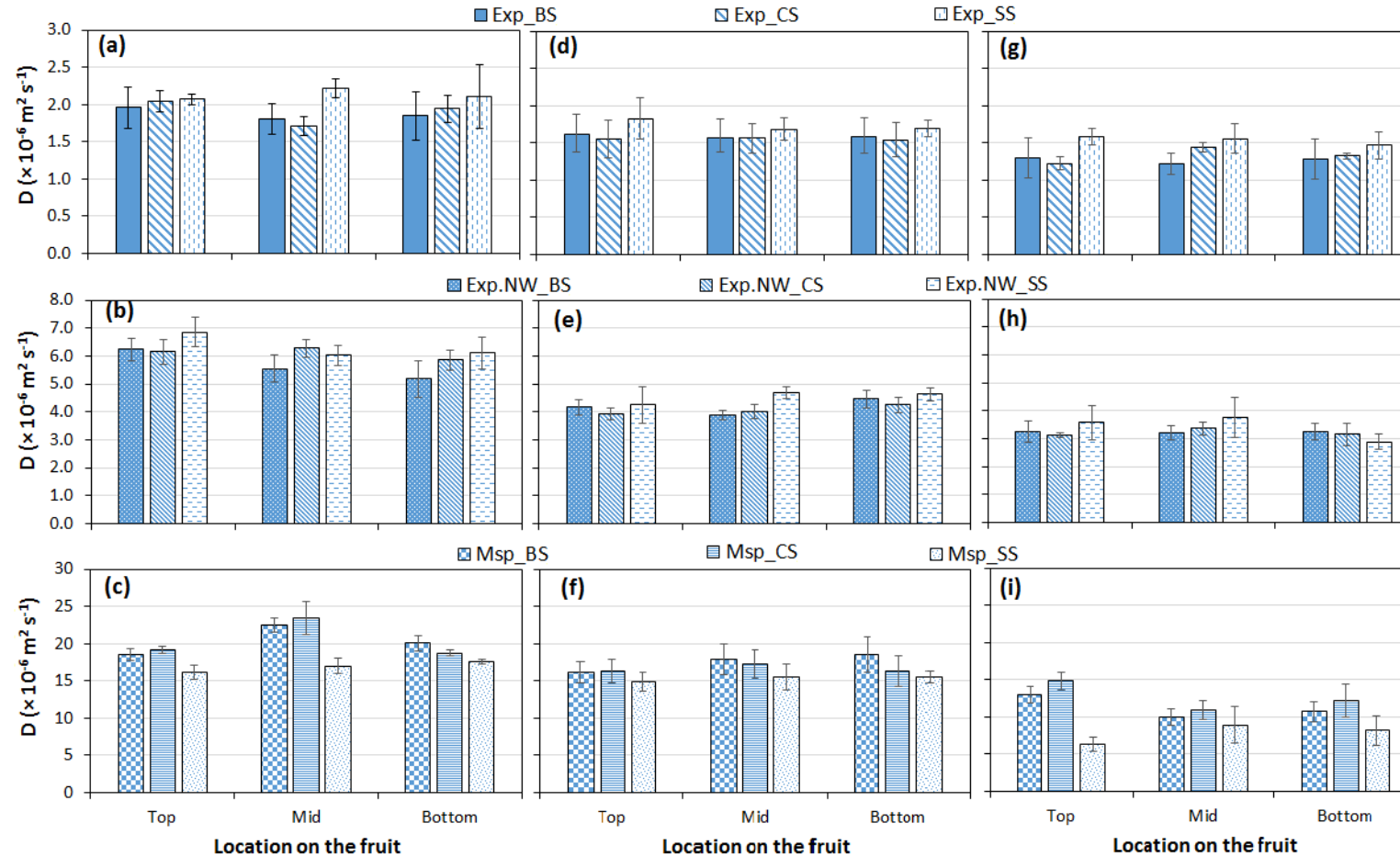


Figure 6.3. Moisture diffusivity (D) of different tissue types excerpted from the top, mid and bottom locations on the pomegranate fruit (cv. Wonderful). The diffusivity of exocarp (Exp), exocarp with no waxy layer (Exp.NW) and the mesocarp (Msp) tissues was determined before storage (BS), after 42 d of cold storage (CS) at 7 °C/90 % RH and after 8 d of shelf storage (SS) of fruit at 23 °C/58 % RH. In each case, D was investigated at three temperatures of 23 °C (a-c), 15 °C (d-f) and 7 °C (g-i). The bars represent mean values of four replicates and the vertical lines are error bars at $P \leq 0.05$.

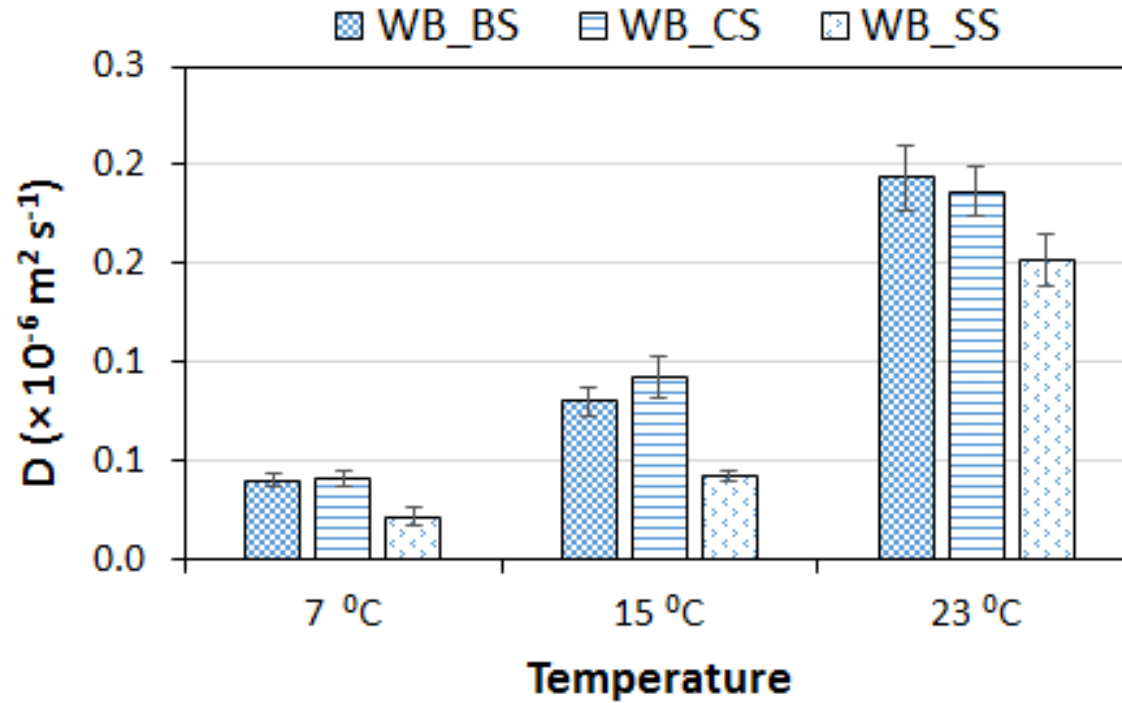


Figure 6.4. Moisture diffusivity (D) of the white membrane (inner epidermal membrane) excerpted from pomegranate fruit (cv. Wonderful). Diffusivity was determined before storage (BS), after 42 d of cold storage (CS) at 7 °C/90 % RH and after 8 d of shelf storage (SS) of fruit at 23 °C/58 % RH. In each case D was investigated at three temperatures of 23 °C (a-c), 15 °C (d-f) and 7 °C (g-i). The bars represent mean values of four replicates and the vertical lines are error bars at $P \leq 0.05$.

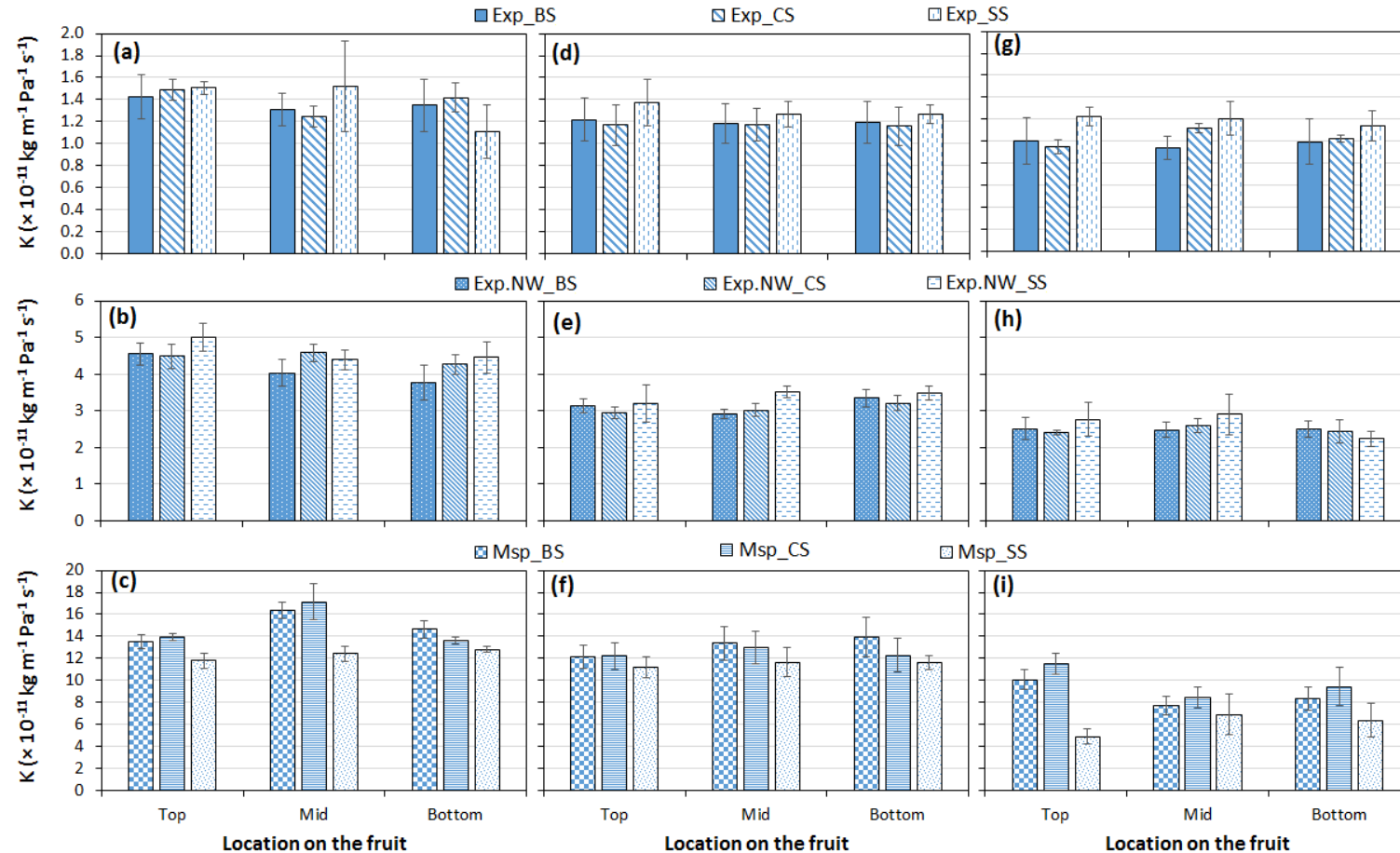


Figure 6.5. Moisture permeability (K) of different tissue types excerpted from the top, mid and bottom locations on the pomegranate fruit (cv. Wonderful). The permeability of exocarp (Exp), exocarp with no waxy layer (Exp.NW) and the mesocarp (Msp) tissues was determined before storage (BS), after 42 d of cold storage (CS) at 7 °C/90 % RH and after 8 d of shelf storage (SS) of fruit at 23 °C/58 % RH. In each case K was investigated at three temperatures of 23 °C (a-c), 15 °C (d-f) and 7 °C (g-i). The bars represent mean values of four replicates and the vertical lines are error bars at P ≤ 0.05.

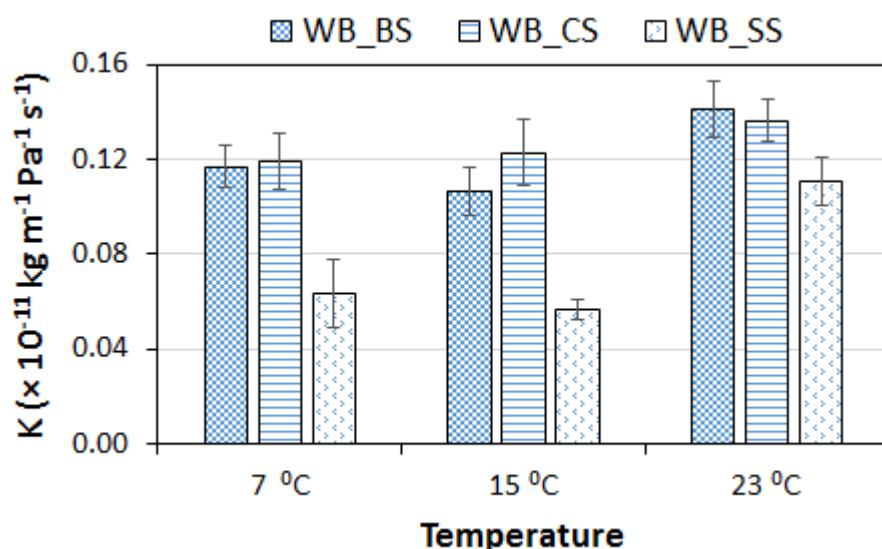


Figure 6.6. Moisture permeability (K) of the white membrane (inner epidermal membrane) excerpted from pomegranate fruit (cv. Wonderful). Permeability was determined before storage (BS), after 42 d of cold storage (CS) at 7 °C/90 % RH and after 8 d of shelf storage (SS) of fruit at 23 °C/58 % RH. In each case K was investigated at three temperatures of 23 °C (a-c), 15 °C (d-f) and 7 °C (g-i). The bars represent mean values of four replicates and the vertical lines are error bars at $P \leq 0.05$.

6.3.3 Effect of tissue location on the fruit

Location on the fruit did not significantly influence moisture diffusivity and permeability among the different tissues. However, D and K were observably higher in the top position (near the calyx) and mid position (equatorial region) than in the bottom position (near the pedicel) especially in the exocarp tissues (**Figure 6.3 and 6.5**). The D of $1.96 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$ was observed in the exocarp tissues from the top location compared to 1.80 and $1.85 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$ in the mid and bottom locations at 23 °C before storage, respectively. Likewise, a K of $1.43 \times 10^{-11} \text{ kg m}^{-1} \text{ Pa}^{-1} \text{ s}^{-1}$ was observed in the top as compared to 1.31 and $1.35 \times 10^{-11} \text{ kg m}^{-1} \text{ Pa}^{-1} \text{ s}^{-1}$ in the mid and bottom locations, respectively. This is probably due to a higher tissue porosity and or higher count of surface openings (lenticels) in the tissues of the top and mid locations as compared to the tissue in the bottom location. Therefore, these results suggest the need to investigate the microstructure of the pomegranate fruit to facilitate the understanding of transport dynamics within the tissues. These results are important in the optimisation of surface waxing applications by differential treatments on the top, middle and bottom of the fruit to

minimise moisture loss and to prevent the production of fermentative off flavours commonly associated with most waxing applications (Barman *et al.*, 2011).

6.3.4 Effect of storage duration

The effective moisture diffusivity varies with harvest maturity being higher in tissues of pear fruit picked at a late date than fruit picked at an early date (Nguyen *et al.*, 2006). Furthermore, the authors observed that the effect of storage time remained unclear due to large biological variability. However, in the current study, the influence of storage on water transport properties was significant across all tissue types and temperatures of determination. Generally, moisture diffusivity and permeability at the end of cold storage was comparably similar to that before storage irrespective of the tissue type, tissue location and temperature of determination. This implies that the 42 d of cold storage were not sufficient to cause a change in the tissue transport properties. Likewise, Verstreken *et al.* (1998) reported a no significant change in the apple flesh diffusivity during ultra-low oxygen storage of apple fruit (cv. Jonagold). However, in the present study, an increase in D and K was observed in the exocarp tissues after the eight days of fruit shelf storage. For example, at 15 °C moisture diffusivity in exocarp tissues increased from $1.57\text{--}1.62 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$ before fruit storage to $1.69\text{--}1.83 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$ after eight days of shelf storage. This increase is attributed to the increase in tissue porosity because of moisture loss. Furthermore, the increase in membrane permeability with storage duration can be attributed to a loss in membrane integrity (Puthmee *et al.*, 2013).

On the other hand, a decrease in D and K was observed in the mesocarp tissues across all temperatures. The eight days of shelf storage resulted in an 8-16 % decrease in moisture diffusivity of the mesocarp tissue from $16.18\text{--}18.52 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$ to $14.88\text{--}15.50 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$ at 15 °C before fruit storage. This is attributed to the tremendous decrease in tissue porosity resulting from excessive moisture loss and shrinkage.

6.3.5 Effect of temperature of determination

Temperature is one of the most important factors influencing moisture transport across fruit tissues as observed in this study. Averagely, moisture diffusivity was 1.97, 6.02, $19.23 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$ and permeability was 1.38, 4.39, 14.03 and $0.13 \times 10^{-11} \text{ kg m}^{-1} \text{ pa}^{-1} \text{ s}^{-1}$ at 23 °C in the exocarp, exocarp with no wax, mesocarp and white membrane tissues, respectively. Decreasing temperature from 23 to 15 °C reduced moisture diffusivity by 17.3, 29.4, 14.3 and 59.71 % and permeability by 11.2, 27.3, 11.8 and 26.4 %, respectively. Further decrease in

temperature from 15 to 7 °C reduced diffusivity by 15.3, 22.9, 36.0 and 52.4 % and permeability by 12.6, 20.5, 34.0 and 24.7 %, respectively. In pear fruit tissues (cv. Conference) increasing temperature from 1 to 20 °C increased the diffusion coefficients by a factor of 2.0 and 3.6 – 9.6 in the cuticle and inner cortex tissues, respectively (Nguyen *et al.*, 2006). In a quite similar situation, higher temperatures are reported to accelerate the movement of water molecules resulting in higher values of effective diffusivity and accelerated drying of banana tissues (Thuwapanichayanan *et al.*, 2011). The results in the current study emphasise the importance of maintaining a cold chain as a very crucial consideration in the minimising of water loss in fresh fruit along the value chain.

6.4 Conclusion

The current study aimed to determine the water transport properties (diffusivity and permeability) of the non-edible pomegranate fruit tissues. The study was carried out on the exocarp/epicarp (outer peel), mesocarp (inner peel) and white membrane (inner epidermal membrane) of the ‘Wonderful’ cultivar which is the most importantly grown and exported in South Africa. Furthermore, the effect of tissue location on the fruit, storage conditions and temperature of determination were investigated.

The study revealed that the main experimental factors of storage duration, fruit tissues type and temperature of determination had a significant influence on the water transport properties (moisture diffusivity and permeability). The interaction between storage duration and tissue type as well as between temperature of determination and tissue type significantly influenced the moisture diffusivity and permeability. Furthermore, the combined effect of storage duration, temperature of determination and tissue type had a significant impact on the transport properties of the tissue. However, tissue location on the fruit as well as its combined influence with our effects did not significantly influence water transport properties.

Across all tested conditions, moisture diffusivity and permeability were lower in the exocarp tissues than in the mesocarp tissues of the peel. However, removing the surface waxy layer greatly and significantly increased the transport properties of the exocarp tissue. These results demonstrate the significant role of the exocarp (outer peel) and the outermost waxy layer in minimising water loss during fruit storage. Therefore industrial management practices such as the washing of the fruit and bulk storage into bins that compromise on the integrity of the exocarp and the waxy layer could result in excessive moisture loss during fruit storage. Very low moisture diffusivity and permeability were observed in the white membrane as

compared to the exocarp and mesocarp tissues. These findings suggest the white membrane as a very protective tissue (highest resistance) against moisture loss from the edible portion (arils) of the fruit. This white membrane (inner epidermis) is the tissue that directly covers the arils (edible portion). These findings further explain why water loss in pomegranate fruit is primarily and majorly from the peel (exocarp and mesocarp) as compared to the arils (edible portion). Therefore, despite the fact that pomegranate fruit are highly susceptible to moisture loss across the numerous pores of the surface of the peel, the edible portion of the fruit is largely preserved from excessive moisture loss by the inner epidermal membrane.

These moisture diffusivity and permeability properties of the fruit tissues are very important in modelling and simulation of fruit storage, processing and preservation processes, designing of efficient packaging, formulation and implementation surface waxing and coating. However, a big knowledge gap exists in relating the water movements and the microstructure of this complex fruit. Therefore, there is a need to investigate the microstructure of the pomegranate fruit to facilitate the understanding of transport dynamics within the tissues.

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SECTION III

Qualitative examination of the microstructure of pomegranate peel using scanning electron microscopy, confocal laser scanning microscopy and x-ray micro computed tomography (Chapter 7 & 8).

CHAPTER 7

IDENTIFICATION AND CHARACTERISATION OF THE PEEL SURFACE STRUCTURES OF POMEGRANATE FRUIT (CV. WONDERFUL) DURING STORAGE

With regard to Chapter 7, pages 171–207, the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Research, data collection, and analysis, image processing, and writing of chapter	80

The following co-authors have contributed to Chapter 7, pages 171–207:

Name	e-mail address	Nature of contribution	Extent of contribution (%)
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Declaration with signature in possession of candidate and supervisor Signature of candidate	26/02/2020 Date
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Declaration by co-authors

The undersigned hereby confirm that:

1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 7, pages 171–207,
2. no other authors contributed to Chapter 7, pages 171–207 besides those specified above, and
3. potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 7, pages 171–207 of this dissertation.

Signature	Institutional affiliation	Date
Declaration with signature in possession of candidate and supervisor	Department of Horticultural Sciences, Stellenbosch University	26/02/2020
Declaration with signature in possession of candidate and supervisor	Department of Horticultural Sciences, Stellenbosch University	26/02/2020

Chapter 7

7 Identification and characterisation of the peel surface structures of pomegranate fruit (cv. Wonderful) during storage

Abstract

Pomegranates are considered luxurious fruit that sell well in the higher market segment. However, the fruit is classified as highly perishable despite its relatively lower respiration rate. The fruit is specifically prone to moisture loss irrespective of its thick rind and tough leathery outer skin, resulting in compromised visual appearance and financial loss. A knowledge gap exists on the microstructure of the fruit, which is very important in understanding water relations regarding the fruit. The study aimed to identify and characterise the surface structures of the pomegranate peel with respect to location on the fruit during storage, to aid strategic weight loss control. The fruit were stored at 7 °C and 90 % RH for 42 d and thereafter transferred to shelf conditions of 23 °C and 58 % RH for eight days. Peel samples were obtained from the top (calyx end), mid (equatorial region) and bottom (pedicel end) locations on randomly selected fruit. Lenticels, micro-cracks and wax layer patterns were examined under scanning electron microscope while the thickness of a waxy cuticle was examined under a confocal laser scanning microscope. The overall peel thickness and peel fractions (exocarp and mesocarp) thickness were monitored using digital Vernier callipers. A higher count of lenticels, larger lenticel size and a generally low peel thickness were observed in the top and mid locations as compared to the bottom locations of the fruit. This suggests that the pomegranate fruit were more susceptible to moisture loss at the top and mid locations as compared to the bottom location. A decreasing profile of the waxy cuticle thickness, increased fragmentation of waxy cuticle, widening and deepening of micro-cracks and a general decrease in peel thickness were observed during fruit storage.

7.1 Introduction

The revived interest in this ancient fruit Pomegranate (*Punica grantum* L) has resulted in increased production, consumption and intensified research owing to its health and nutritional benefits (Aviram *et al.*, 2008; Guo *et al.*, 2008; Arendse *et al.*, 2014). Pomegranates are

considered luxurious fruit that sell well in the higher market segment (CBI, 2019) and there has been a growing demand for high quality, healthy and exotic fruit both for fresh use and for processing into juices and other products (Seeram *et al.*, 2006; CBI, 2019). Current global production is estimated at three million tonnes annually (Erkan and Dogan, 2018) with the majority cultivation carried out in the Northern Hemisphere and Europe providing the largest global market (CBI, 2019). South Africa pomegranate production is estimated at 7,337 tonnes with 69 % of the fruit as exports (NAMC, 2017) and the country's exports are expected to increase to over 8,170 tonnes by 2020 (POMASA, 2018).

However, the fruit is classified as highly perishable (Barman *et al.*, 2011) despite its relatively lower respiration rate during postharvest storage (Caleb *et al.*, 2012). The fruit is specifically prone to moisture loss owing to the relatively numerous micro openings on the peel, irrespective of its thick rind and tough leathery outer skin (Opara *et al.*, 2010; Fawole and Opara, 2013). A weight loss of 4-5 % is considered sufficient to cause shrivelling of the fruit (Elyatem and Kader, 1984; Lufu, 2017). Even in the cases of no visible shrivelling, water loss can undesirably affect the visual appearance, flavour and textural properties of the fruit (Pareek *et al.*, 2015). Excessive weight loss results into browning of the peel and arils, as well as hardening of the rind (Kader *et al.*, 1984; Artés *et al.*, 2000; Caleb *et al.*, 2012).

The peel of most fruit consists of several layers which play important roles in protecting against mechanical damage, penetration of pathogens, and prevention of excessive transpiration while facilitating adequate gaseous exchange with its surrounding, before and during storage (Lara *et al.*, 2014; Wang *et al.*, 2017). In most fruit such as apples, the peel is entirely the exocarp covering the entire fruit. The exocarp is further differentiated into the inner layer known as the hypodermis and the outer layer, the epidermis (Homutová and Blažek, 2006). The epidermis is often overlaid with a non-cellular layer called the cuticle. The cuticle consists of cutin layer and a lining of the epicuticular wax layer (Veraverbeke *et al.*, 2001a). It is the waxy part of the cuticle that is responsible for the big resistance against transpiration, majorly due to the hydrocarbons, long-chain alcohols and aldehydes of the wax (Possingham *et al.*, 1967; Veraverbeke *et al.*, 2003). The cuticle often covers other openings such as stomata, lenticels and micro-cracks on the epidermal layer and commonly extends through the epidermal cells in some parts (Konarska, 2012). These openings play a fundamental role in the water vapour permeance and moisture loss across the fruit surface (Maguire *et al.*, 1999).

The availability of different imaging techniques has facilitated the increasing interest to understand the structure of the peel of various fruit which is very vital in furthering the understanding of moisture dynamics within and outside the fruit. Light microscopy (LM), scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) have been applied to examine and characterise the morphology and structure of fruit skin and surface structures such as wax, micro-cracks and stomata in plums (Konarska, 2015; Kritzinger and Lötze, 2019) and apples (Maguire *et al.*, 1999; Veraverbeke *et al.*, 2001a; Konarska, 2012; Singh *et al.*, 2016; Yang *et al.*, 2017).

However, there is still very limited information on the surface microstructure of fruit with thick peels technically referred to as the rind such as in pomegranate and citrus fruit. The rind of this berry type of fruit consists of the hard outer pericarp (exocarp) and spongy inner mesocarp surrounding the arils (Stover and Mercure, 2007). For the first time, the anatomy of pomegranate fruit (cv. Hicaznar) exocarp was examined using a light microscope (Yazici *et al.*, 2011). The authors observed that the exocarp of the pomegranate peel consists of the epidermis as the outer most cell layer on the peel structure lying immediately underneath a non-cellular cuticular layer. Furthermore, lens-shaped lenticels were observed in the epidermal cell layer of the fruit. Zhang *et al.* (2015) carried out a morphological and histological characterisation of browning on the cultivar ‘Dahongpao’ and observed browning of the regions near stomata and vascular bundles. This current study aimed to identify and characterise the changes in peel surface structures of pomegranate fruit (cv. Wonderful), with the aid of SEM and CLSM.

7.2 Materials and methods

7.2.1 Fruit acquisition

Pomegranate fruit (*Punica granatum* L. cv. Wonderful) at acceptable commercial maturity were carefully and individually harvested by hand from a commercial orchard situated in Porterville, Wellington (33° 38' S, 19° 00' E), Western Cape Province, South Africa. ‘Wonderful’ is the most importantly produced and exported cultivar from the country. The orchard applies the conventional practices of mineral fertilizers, pesticides and irrigation. All fruit were harvested from the central canopy positions on randomly selected trees in the same rows to minimise variation due orchard management practices such as irrigation and fertilizer application and climacteric influence such as wind, rain and sunshine. Care was taken to select

uniformly medium-sized, coloured, intact pomegranate fruit. Fruit were transported in ventilated plastic trays cushioned with paper pads to the postharvest research laboratory, Stellenbosch University. Fruit were closely inspected to ensure that they were free from surface defects such as cracks and then packed in dozens inside single layer display type paper cartons, cushioned with paper trays at the bottom. Throughout all stages of harvesting, transportation and sample preparation, extra care was taken to avoid rubbing of fruit surfaces intended for microscopic observation, thus ensuring the preservation of natural waxing of the fruit peels.

7.2.2 Experimental design

A total of 45 fruit in four cartons were used in the study. The fruit were stored at 7 °C and 90 % RH for 42 d and thereafter transferred to shelf conditions of 23 °C and 58 % RH. This was to mimic the maximum sea freight duration of pomegranate fruit from South Africa to Europe across the Atlantic Ocean, followed by open shelf marketing and storage before consumption. Fifteen fruit were randomly selected before storage, after 42 d of cold storage and again after an additional eight days of shelf storage. Five fruit were used in the microscopic analysis and the remaining ten fruit in the monitoring of peel layer thickness, at each sampling time.

7.2.3 Sample preparation

7.2.3.1 Micro-examination of surface structures with SEM

The fruit were left to condition at room atmosphere for two hours. A cuboid-shaped sample of dimensions $7 \times 7 \times 3$ mm each, was excised from the top (near the calyx), middle/mid (equatorial region) and bottom (near the pedicel) locations on each of the five fruit (**Figure 7.1a**) using sharp blades. The cuboids were fixed by submerging them in a 2.5 % glutaraldehyde solution buffered with 0.1 M phosphate buffer and stored at 4 °C overnight. Samples were washed three times for ten minutes in 0.1 M phosphate buffer (at a pH of 7.2). Post-fixation was carried out with 1 % osmium tetroxide in 0.1 M phosphate buffer at room temperature in light-proof vials for two hours. Osmium tetroxide has good fixative and excellent stain abilities for lipids in membranous structures. Samples were then washed three times for ten minutes with distilled water. The samples were slowly dried between filter paper in an oven at 37 °C for 48 hours, instead of the conventional dehydration using ethanol and hexamethyldisilazane (HMDS) to minimise the destabilisation and loss of the natural surface wax. The dehydrated samples were mounted on metal specimen stubs, sputter-coated with gold for 3.5 mins using a sputter coater to improve electron emission of the samples.

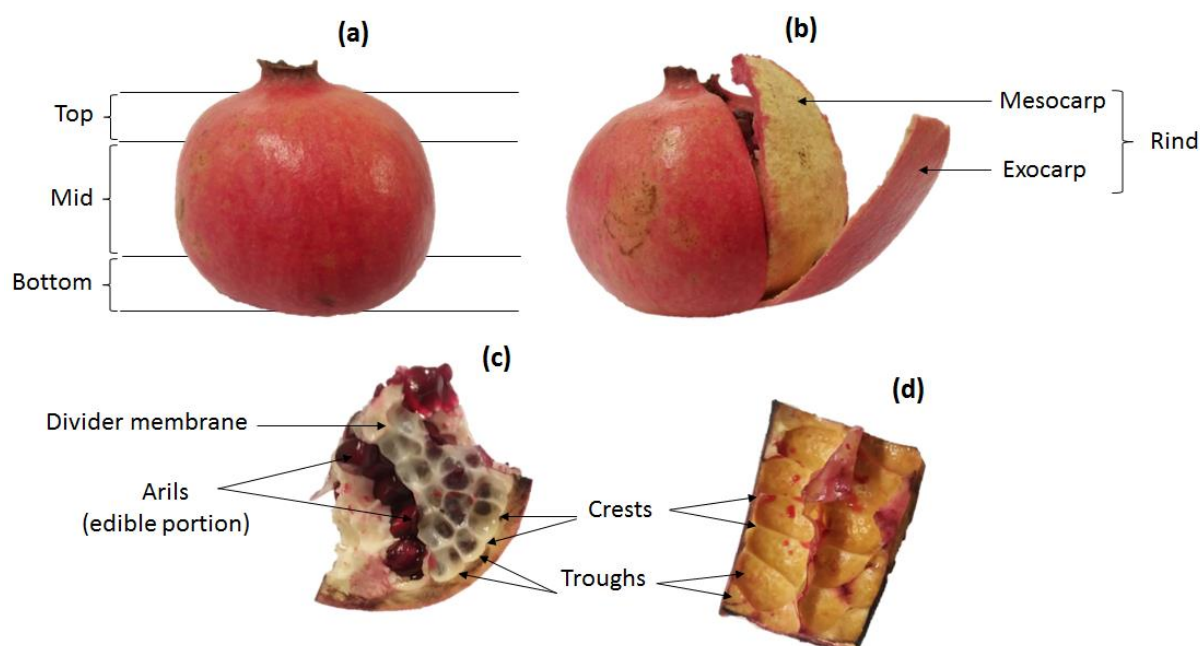


Figure 7.1 Fractions of the pomegranate fruit peel. Locations (top, mid and bottom) of sampling (a), outer (exocarp) and inner (mesocarp) peel fractions (b), fruit segment exposing the arils and the divider membrane (inner epidermis) (c) and dehydrated fruit peel after shelf storage for 8 d at 23 °C/58 % RH (d).

7.2.3.2 Micro-examination of waxy cuticle with CLSM

A stock solution of 0.5 mg mL⁻¹ of Nile red (9-diethylamino-5H-benzo[α]phenoxazine-5-one) in 100 % ethanol was prepared, filtered through a 0.22 μ m syringe filter to reduce fluorescent background and stored at -20 °C until required. A fresh working solution was prepared each time before imaging by diluting 20 μ l of the stock solution in 980 μ l of phosphate-buffered saline (PBS). A thin slice of about 1 mm thickness was longitudinally hand sectioned from the top, mid and bottom locations on each of the five fruit using sharp blades. The samples were individually stained with about 15 μ l of fresh Nile red working solution for 20 minutes. The aim was to stain the wax layer of the fruit peel. Nile red is a lipophilic stain capable of staining the intracellular lipid droplets and can fluorescent with varying colours of deep red to strong yellow-gold emission in a lipid-rich environment.

7.2.3.3 Macro-examination of peel fractions

The fruit were individually cut along the vertical axis into two halves using a sharp hand knife. Peel segments were then sectioned from the top, mid and bottom locations (**Figure 7.1a**) along the opposite side of the two fruit halves using sharp specialised sectioning razor blades. Furthermore, the exocarp (the outer peel) fraction of each peel (rind) section was carefully

separated from the mesocarp (the inner peel) fraction using the sharp sectioning blades (**Figure 7.1b**).

7.2.4 Qualitative analysis

7.2.4.1 Scanning electron microscopy

The sputter-coated samples prepared for micro-examination of surface structures were examined using field emission scanning electron microscope (Merlin Zeiss GeminiSEM 500, Germany). The samples were scanned under a vacuum of 10^{-5} Pa and using an accelerating voltage of 30 kV. Several images of varying magnification (50-1000 \times) were obtained per sample. Images taken at low magnification were used in the general counting of surface structures while the detailed images taken at high magnification were used for specific characterisation of individual surface structures.

7.2.4.2 Confocal laser scanning electron microscopy

The samples prepared for micro-examination of waxy cuticle were mounted and clipped in between clean glass microscope slides and observed under a Carl Zeiss Confocal LSM 780 Elyra PS1 (Carl Zeiss, Germany) with SR-SIM and PALM/dSTORM super-resolution platforms for multiple colour analysis. The microscope is fitted with an argon multiline laser 25 mW at 458 nm, 488 nm and 514 nm, 561 nm and 633 nm lasers. The beam splitter used was MBS 488/561/633 (main beam splitter, reflecting at 488, 561 and 633 nm). The fluorescence of Nile red was visualised by excitation at 515-530 nm wavelength and detection at 650 nm LP. Specimens were photographed with an Andor EM-CCD camera IXon DU 885 using a combination of emitted and transmitted light. During image acquisition, each line was scanned eight times and averaged to minimise background noise. The obtained images were projected from the image stacks in the Z-direction using the maximum intensity module of the ZEN 2011 imaging software (Carl Zeiss, Germany).

7.2.5 Quantitative measurements and calculations

7.2.5.1 Lenticels and micro-cracks

The obtained SEM images were further processed using ImageJ software and micro-measurements were carried out on the surface structures. The counting of open lenticels, closed lenticels, total lenticels (open and closed) and micro-cracks was done on images of a surface area of about 4.1 mm² and results presented as count per square millimetre (counts mm⁻²).

Using a freehand selection tool, the edge of each surface opening was carefully traced out. The surface area of each opening was automatically calculated. The fraction of total open area and closed lenticels were calculated from **Equation 7.1** and **7.2**, respectively.

$$TO_A = \frac{(TOL_A + TMC_A)}{I_A} \times 100 \quad (7.1)$$

$$CL_A = \frac{TCL_A}{I_A} \times 100 \quad (7.2)$$

Where; TO_A and CL_A are percentages of total open area and closed lenticels area, TOL_A is total open lenticels area, TCL_A is total closed lenticels area, TMC_A is total micro-cracks area and I_A is image area.

The diameter and shape characteristics such as porosity, circularity and roundness of each open lenticel were calculated. The Feret's diameter (maximum calliper) was calculated as the longest distance between any two points along the selected edge. Porosity, circularity and roundness were calculated from **Equation 7.3**, **7.4** and **7.5**, respectively.

$$\emptyset_{OL} = \frac{OL_A}{CA} \times 100 \quad (7.3)$$

$$C_{OL} = 4\pi \times \frac{OL_A}{(OL_P)^2} \times 100 \quad (7.4)$$

$$R_{OL} = 4 \times \frac{OL_A}{\pi \times (M_1)^2} \times 100 \quad (7.5)$$

Where \emptyset_{OL} (%) is porosity of open lenticel, OL_A (μm^2) is open lenticel area, CA (μm^2) is convex area of the lenticel, C_{OL} (%) is circularity of open lenticel, OL_P (μm) is perimeter of open lenticel, R_{OL} (%) is roundness of open lenticel and M_1 (μm) is the major axis. A greater porosity close to 100 % indicates greater openness of the lenticel to facilitate transport. A circularity and roundness of 100 % indicate a perfect circle while values closer to zero indicate an increasingly elongated shape.

7.2.5.2 Thickness of waxy cuticle

Micro-measurements of the thickness of the non-cellular waxy cuticle layer was carried out using ImageJ software on the images obtained from CLSM. The thickness of the surface wax cuticle was measured as the vertical perpendicular depth of the brightly fluorescent waxy cuticle, while the intra-layer wax thickness was measured as the brightly fluorescent projections into the epidermal layer at the surface of the peel. In each case, measurements were taken at five randomly selected locations along the length of the peel image, therefore results were presented as means of 25 readings from the replicates per fruit position per sampling time.

7.2.5.3 Thickness of macro peel fractions

The thickness of the macro peel fractions was carried out on ten fruit using a digital Vernier calliper (Mitutoyo, Kawasaki, Japan, ± 0.01 mm). The thickness of the overall fruit peel, exocarp and mesocarp were then measured at four different points along the length of the section. For the case of the overall fruit peel and mesocarp thickness, two of the measurements were taken at the crest points in between the arils and the other two at the trough points directly below the arils to cater for the variation (**Figure 7.1C-D**). The thickness of the inner divider membrane (inner epidermis) was also measured per fruit. Sectioning and measurements were carried out on one sample at a time while keeping the rest under cold storage at 4 °C to minimise enzymatic browning upon cutting the peel.

7.3 Results and discussion

7.3.1 Identification of surface structures with SEM

The examination of the pomegranate peel surface with SEM identified several features that are comparable in structure to those found in previous studies carried out on apples, pear and plums (Skene, 1963; Maguire *et al.*, 1999; Veraverbeke *et al.*, 2001a; Konarska, 2015; Yang *et al.*, 2017; Kritzinger and Lötze, 2019). These surface structures have been identified majorly as openings such as lenticels and micro-cracks, besides other structures in the form of deposits and fragments of the waxy cuticle. **Figure 7.2** shows the general appearance of the pomegranate peel surface with visible openings and how they vary with sample locations on the fruit during storage.

Various shapes and sizes of lenticels were also observed from the different locations on the fruit during storage (**Figure 7.3**). Common shapes included round-shaped, oval-shaped, lens-shaped and stomata-like, with the majority being round and stomata-like is appearance. Yazici *et al.* (2011) describe the lenticels observed on the ‘Hicaznar’ cultivar as being predominantly lens-shaped and evenly distributed on the epidermis. The lenticels were easy to identify by observation under high magnification as open lenticel and closed lenticels (clogged with wax or unknown material). The stomata-like lenticels have un-functional guard cells and tend to develop micro-cracks at the ends in between the guard cells. This agrees with previous research that lenticels develop from un-functional stomata (Gibert *et al.*, 2010). Only a few lenticels were observed with no noticeable guard cells. Mycelia were observed growing in some of the lenticel openings.

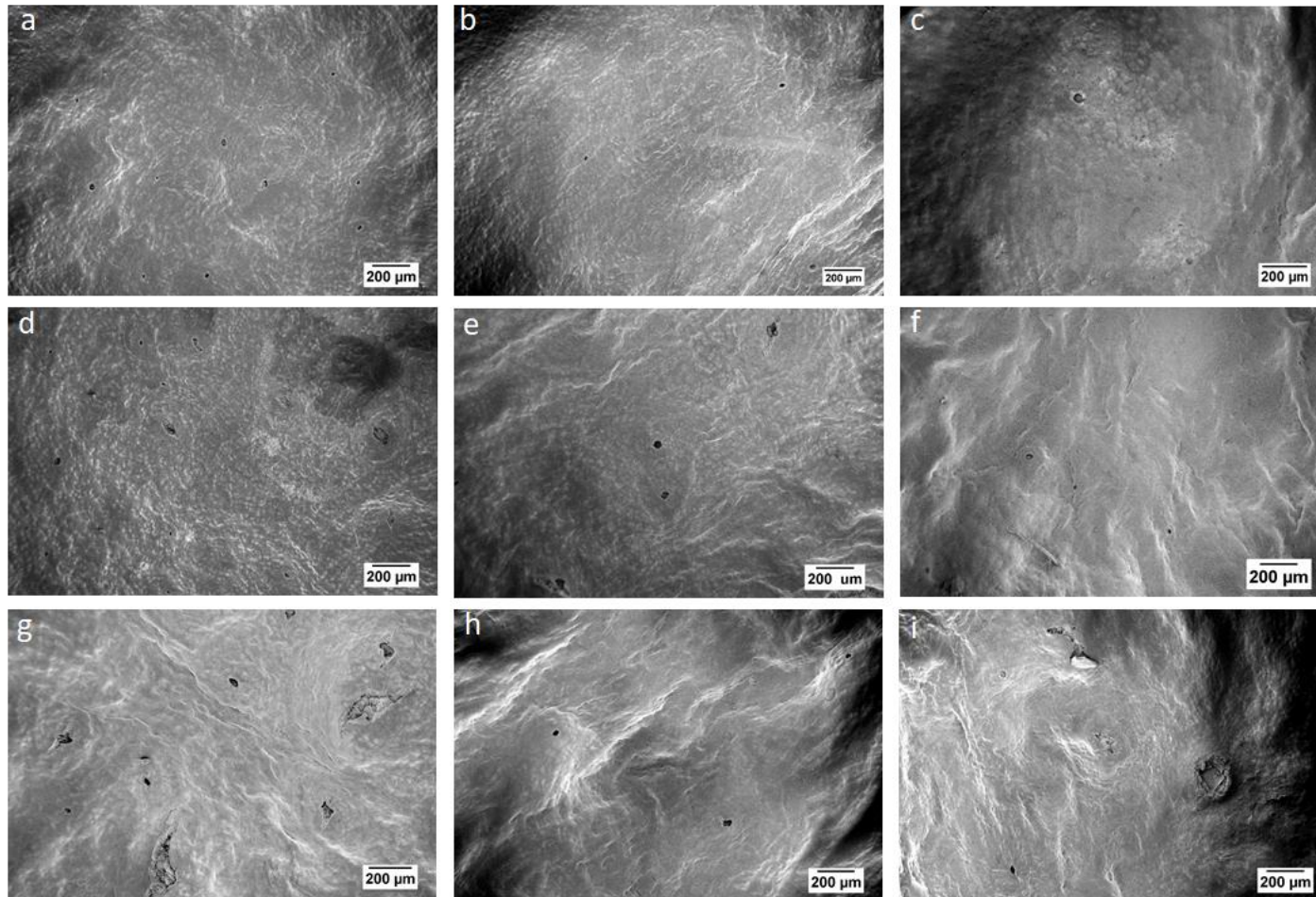


Figure 7.2 Surface openings distribution on the surface of the pomegranate fruit peel (cv. Wonderful) during storage. The scanning electron microscope images were taken from the top (images on the left), mid (centre images) and bottom (images on the right) locations on the fruit before storage (a-c), after 42 d at 7 °C/90 % RH (d-f) and after additional eight days of shelf storage at 23 °C/58 % RH (g-i).

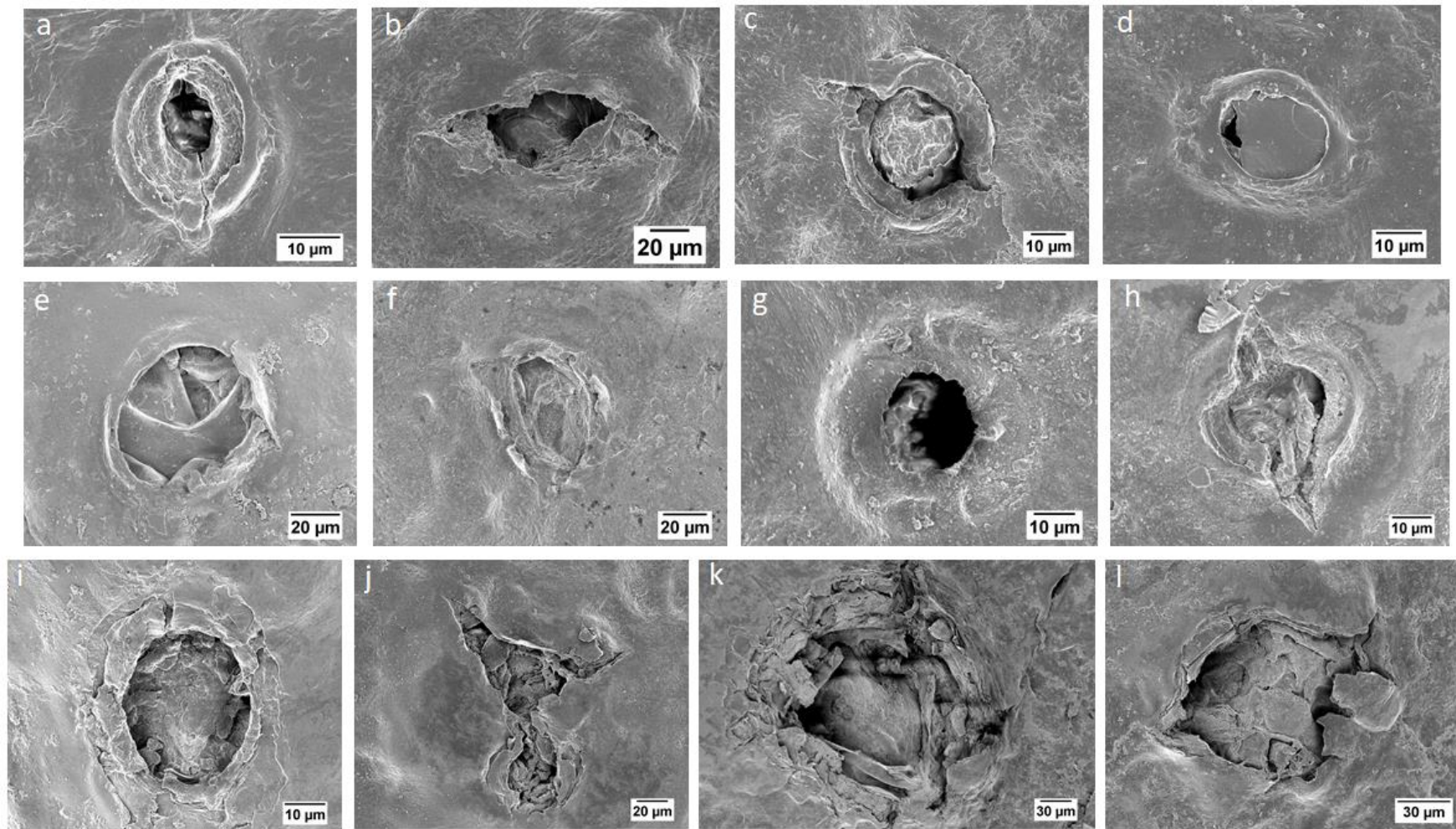


Figure 7.3 Changes in lenticel shape and structure on the surface of the pomegranate fruit peel (cv. Wonderful) during storage. The scanning electron microscope images were taken before fruit storage (**a-d**), after 42 d at 7 °C/90 % RH (**e-h**) and after additional eight days of shelf storage at 23 °C/58 % RH (**i-l**). Oval (**a**), lens (**b**), round (**c-e, g, i**) clogged (**c, e**), open (**a, b, g**) and closed (**f**).

7.3.2 Changes in peel surface structures

7.3.2.1 General open surface area of the peel

Figure 7.4 shows the percentage of the overall total open area at the different fruit surface locations during storage. Generally, a higher open area fraction was observed on the mid location than on the top and bottom locations on the fruit surface and this increased with storage period. Before fruit storage, the top, mid and bottom locations had an open area fraction of 0.4, 0.7, and 0.6 % which increased to 1.1, 1.7 and 0.7 % at the end of the additional shelf storage period, respectively. The respective 2.7, 2.6 and 1.1 times increase could be attributed to widening of surface openings with time as the fruit loses moisture across the peel. On the other hand, the fraction of the observably closed surface openings in the form of closed lenticels was generally low across all tested conditions, especially at the end of the shelf life storage period. It is important to note that different types of surface openings contribute to the overall total open fraction, depending on the maturity stage of the fruit. In our case lenticels and cracks are the most readily available while stomata are least expected as they tend to lose their functionality and turn into lenticels during fruit development and maturity. This phenomenon is especially due to skin expansions and when the guard cells of the stomata lose their ability to control opening and closure during growth and development (Gibert *et al.*, 2010).

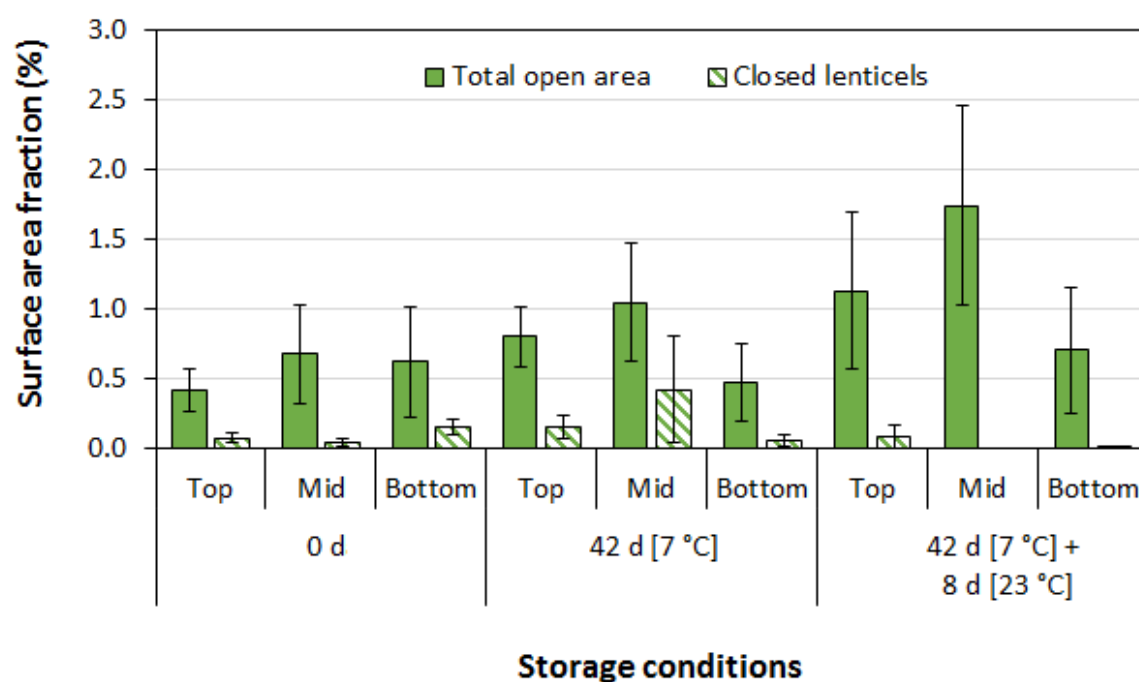


Figure 7.4 Percentage of total open area and closed lenticel area on the surface of pomegranate fruit (cv. Wonderful).

7.3.2.2 Unit count of surface openings

The number of surface openings varied with the location on the fruit. Most of the surface openings were lenticels and micro-cracks. Generally, the highest number of lenticels were observed at the top followed by the mid and then the bottom locations on the fruit (**Figure 7.5**). On average, a total (both open and closed) of about five, three and two lenticels per square millimetres are observed on the top, mid and bottom locations on the fruit, respectively as can be observed in **Figure 7.5a**. These results partly explain the findings from the previous study (**Chapter 6**) where a relatively higher water permeability and diffusivity was observed in the top than in the mid and bottom located samples of the exocarp. This variation can be attributed to natural mechanisms established during tissue development and fruit growth against excessive transpiration, where regions of the leaves and fruits that are more exposed to sunshine tend to have thicker waxy cuticles (Rehman *et al.*, 2015), more compacted cells and fewer surface openings than regions under shade. The pomegranate fruit are orientated on the tree in such that the top (calyx end) faces down towards the ground while the bottom position (pedicel end) faces upwards and is more susceptible to higher sunshine exposure.

On the other hand, relatively higher numbers of lenticels were observed on the fruit surface before storage than at the end of cold storage and shelf life storage (**Figure 7.5b**). This could be attributed to the covering of some surface openings as a result of wax re-distribution under storage conditions. Changes in the composition and distribution of the wax layer and the covering of the cracks and stomata by the wax layer play an important role in the minimising of excessive moisture loss (Veraverbeke *et al.*, 2001b). Quite similar results were observed in climacteric fruit. For instance, Veraverbeke *et al.* (2001a) observed that more cracks were covered with wax for apple fruit (cvs. Elstar, Jonagored and Jonagold) stored under ultra-low oxygen conditions for about 270 d at 1 °C and subsequent shelf storage of 14 d at 20 °C. In our current study, the occurrence of such a phenomenon is further buttressed by the observed decrease in the thickness of the waxy cuticular layer during storage as explained further in **Section 7.3.4** below.

More open lenticels than closed lenticels were observed irrespective of the location on the fruit and storage conditions (**Figure 7.5a-b**). The number of open lenticels was highest at the top (3.5 mm⁻¹), followed by mid (1.9 mm⁻¹) and least on the bottom (1.0 mm⁻¹) locations of the fruit. Furthermore, open lenticels were more before storage and less at the end of the eight-day shelf storage period. The highest number of cracks was observed at the bottom location of

the fruit than at the top and mid locations. Most of the cracking was observed on the fruit before storage.

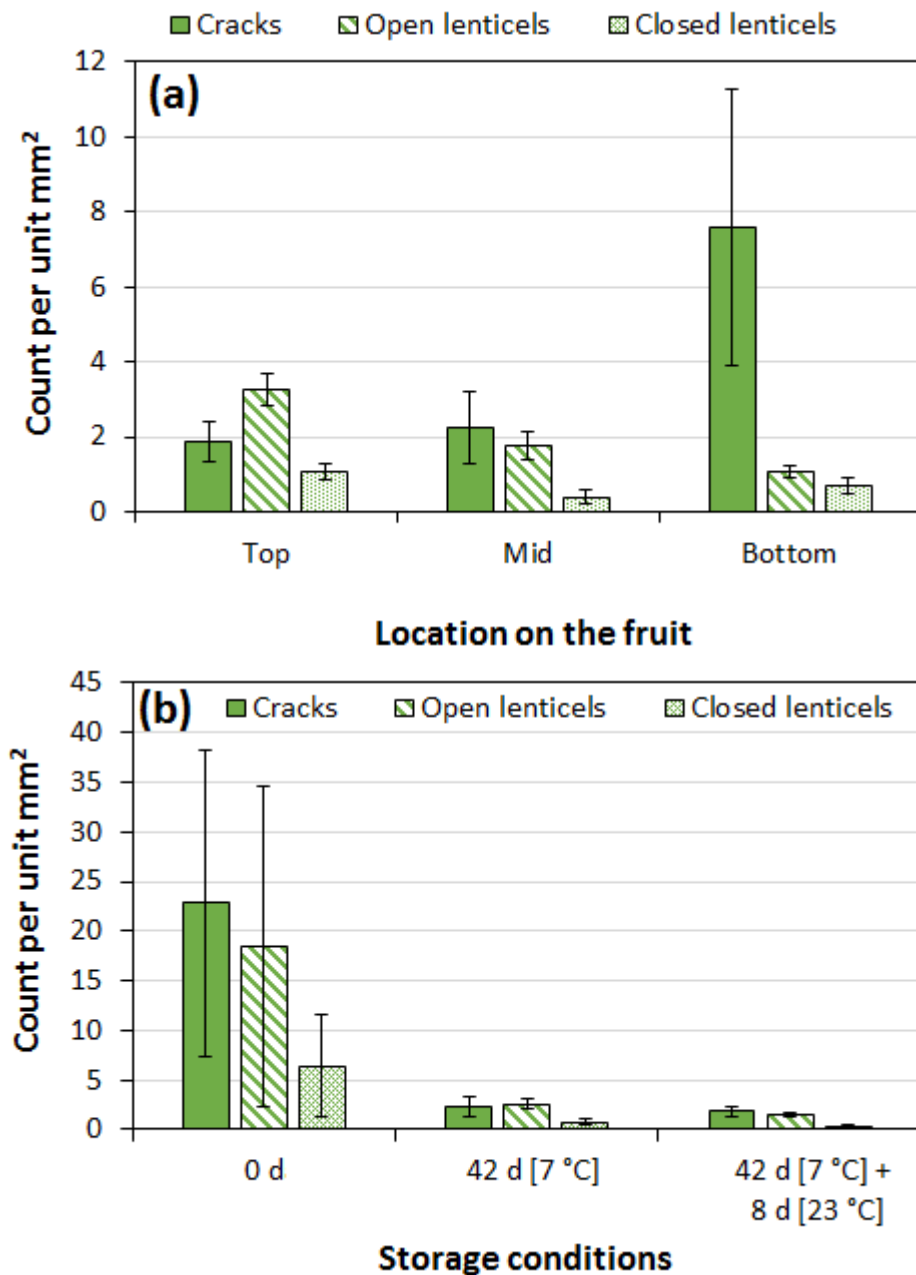


Figure 7.5 Total count of visible cracks, open and closed lenticels per unit surface area of the pomegranate fruit (cv. Wonderful) with respect to locations on the surface (a) and storage conditions (b).

7.3.2.3 Lenticels shape and size descriptors

Lenticels varied greatly in size, shape and degree of opening or clogging with waxy materials as in the previous study on ‘Bluefre’, ‘Sweet Common Prune’ and ‘President’ plum cultivars (Konarska, 2015). Just like stomata, some lenticels are clogged with wax or other materials

such as suberised periderm, thus providing a barrier against water loss (Veraverbeke *et al.*, 2003). The size distribution of the lenticels varied with location on the fruit surface and with storage conditions. **Figure 7.6a-b** shows that generally 96, 84 and 98 % of the lenticels on the top, mid and bottom locations on the fruit surface, respectively were less than 0.01 mm^2 in size. Likewise, 98, 85 and 89 % of the lenticels on the fruit surface before storage, end of cold storage and after shelf storage, respectively were less than 0.01 mm^2 in size. This implies that the lenticel size was more uniform on the top and bottom than on the mid locations and that it decreases with storage time. The lenticels on the mid location were generally larger in size than those at the top and bottom locations and that lenticel size increased with time as the fruit lost moisture (**Figure 7.7a-b**). Lenticel Feret diameter increased by 60.1, 48.1 and 12.8 % from 44.6, 85.6 and $51.7 \text{ }\mu\text{m}$ to 111.7, 164.7 and $59.4 \text{ }\mu\text{m}$ in the top, mid and bottom locations on the fruit surface, respectively by the end of the shelf storage period. Likewise, lenticel surface area increased by 84.6, 69.1 and 55.5 %, in the top, mid and bottom locations, respectively. This suggests that the high overall open area fraction observed in the mid location was mainly due to the larger lenticel size as compared to the lenticel count per unit area.

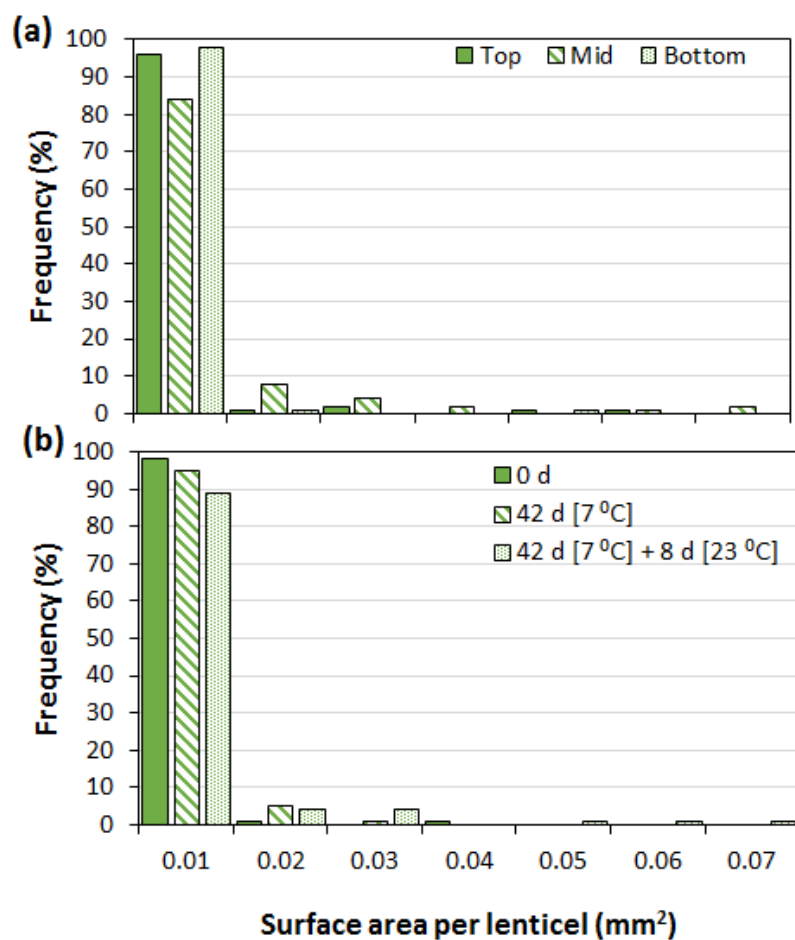


Figure 7.6 Frequency distribution of lenticel surface area on the different surface locations on the fruit (a) under different storage conditions (b).

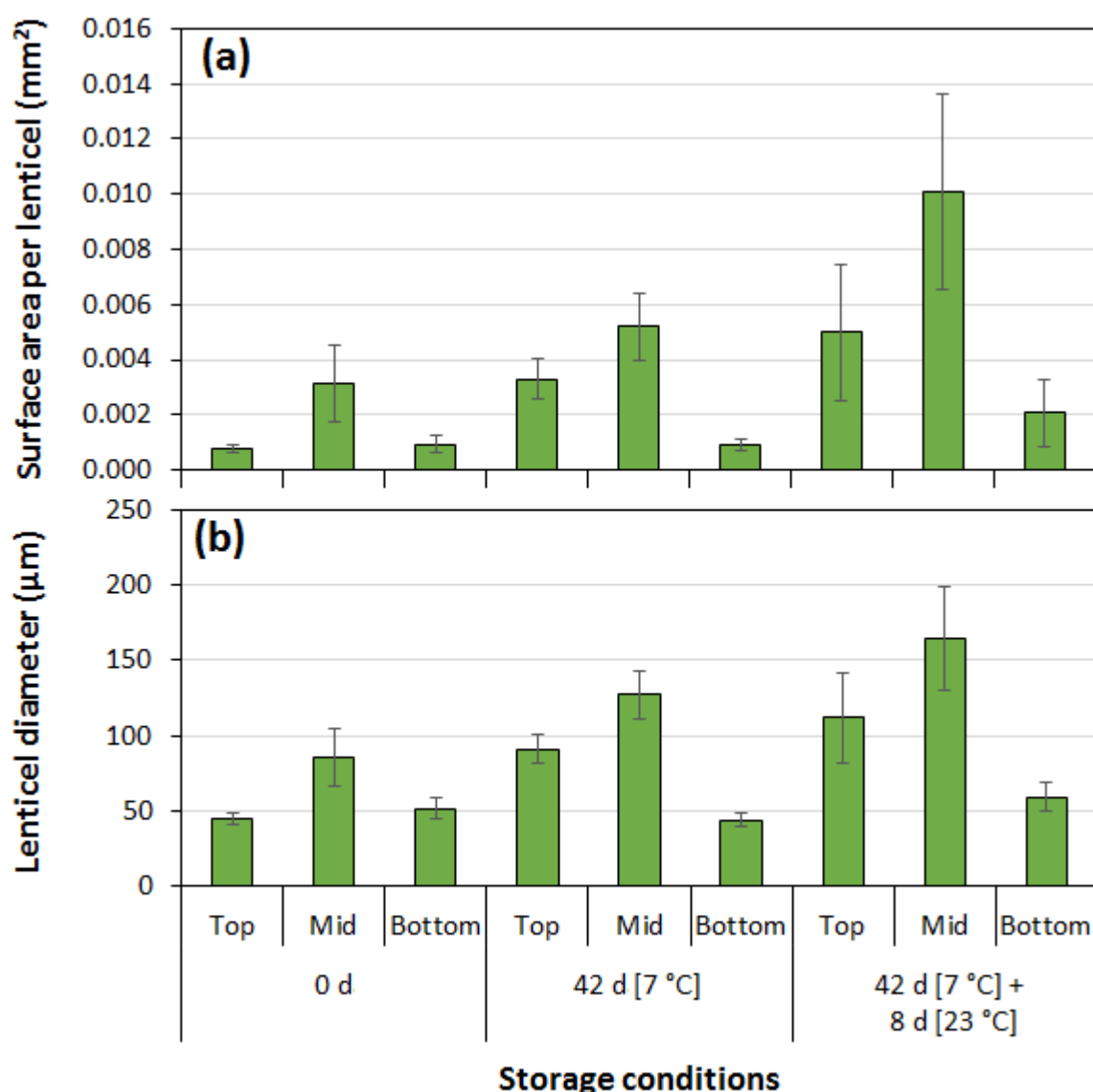


Figure 7.7 Size characteristics of lenticels on the surface of pomegranate fruit (cv. Wonderful). Surface area (a) and Feret diameter (b) of individual lenticels.

The lenticel shape characteristics varied greatly with both location on the fruit surface and storage conditions. Lenticels at zero and 42 d of cold storage had smoother and intact shapes compared to disrupted and rough shapes at the end of the additional eight days of shelf storage as observed earlier in **Figure 7.3**. This is attributed to excessive moisture loss during shelf storage resulting in drying and flaking of the epidermal cells. The porosity of the lenticels were generally higher at the top (86.2 %) location than at the mid (79.8 %) and bottom (80.9 %) locations on the fruit surface (**Figure 7.8a-b**). This implies that the lenticels at the top of the fruit were more open than those at the mid and bottom locations. The porosity of the lenticels decreased slightly from 83.3 to 81.4 % when the fruit were transferred from cold storage conditions at the end of the 42 d to shelf life conditions for eight days. Likewise,

circularity and roundness were higher at the top (63.5 and 53.9 %) than at the mid (53.1 and 50.0 %) and bottom (56.7 and 48.6 %) locations on the fruit surface, implying that the shapes of the lenticels at the top were closer to perfect circles than those at the mid and bottom location. The shape characteristics of the openings are important in the facilitation of fluid flow and hence an easy flow of fluids such as respiratory gases and moisture is more expected at the top of the fruit.

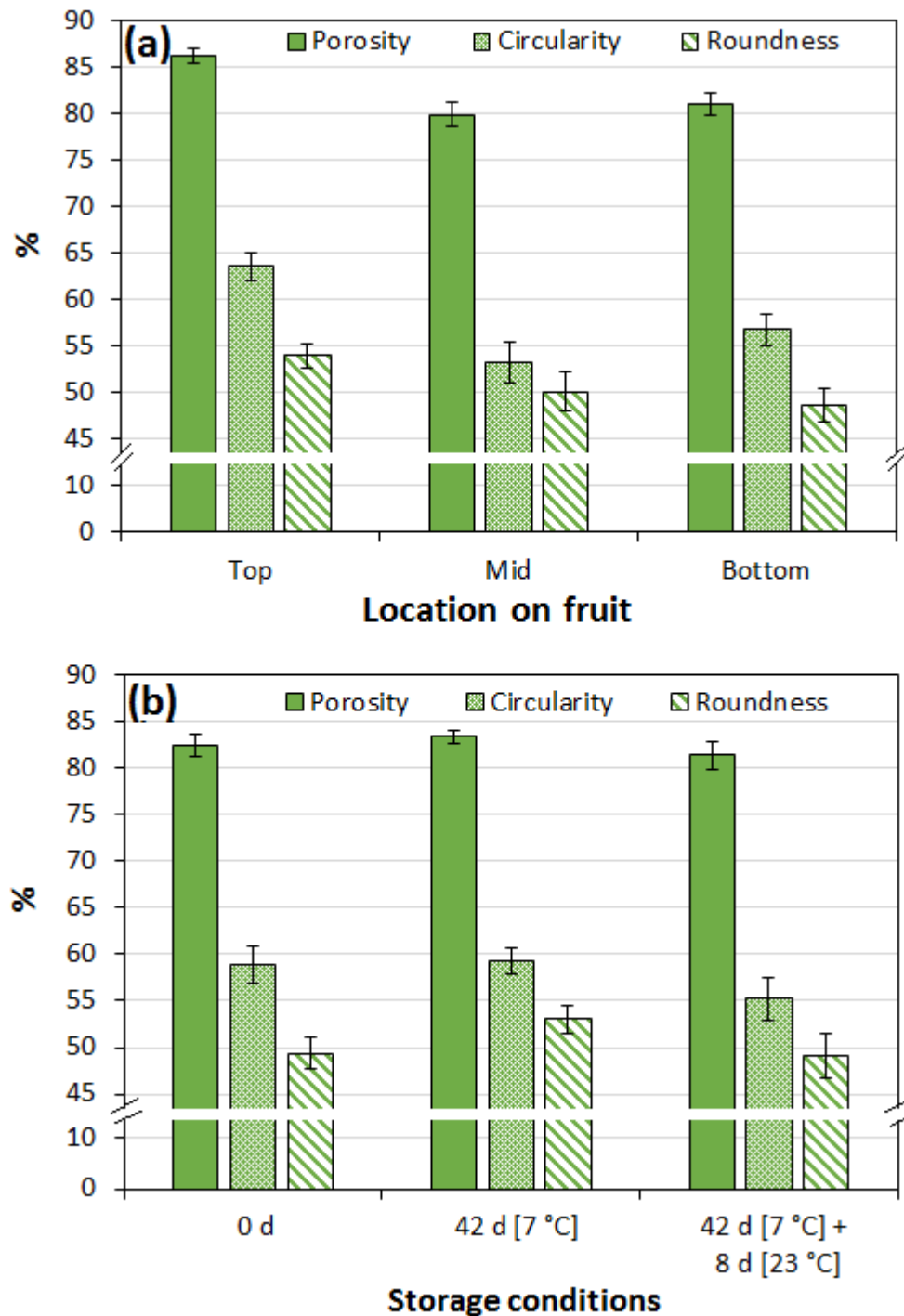


Figure 7.8 Shape factors of lenticels on the surface of pomegranate fruit (cv. Wonderful). Changes with respect to locations on the fruit (a) and storage conditions (b).

7.3.2.4 Micro-cracks characteristics

Micro-cracks appear as cuts, tears or breakages of varying sizes in the epidermal layer of the fruit peel. Some of the micro-crack were covered by the waxy cuticle while wax deposits and mycelia were noticed inside some cracks (**Figure 7.9a-l**). Cracks and tears were more common

at the two ends of most lens shaped lenticels. A comparable situation was reported in plums cultivars both smaller and larger micro-cracks were noticed as extensions along the axis of the guard cells of the stomata of the (Konarska, 2015). The authors also noticed the covering of some micro-cracks under the waxy cuticular layer. The micro-cracks before fruit storage and 42 d of cold storage were shallow and narrow as compared to wide and deep micro-cracks at the end of the additional eight days of shelf storage. The deepening and widening of the cracks at the end of the shelf storage are attributed to skin deformations resulting from excessive moisture loss. Furthermore, the 3D surface plots in **Figure 7.10** revealed that the area of the peel around the micro-cracks was more depressed at the end of shelf storage than before and after cold storage.

Micro-cracking on the peel surface is induced by several factors including genetic influence, orchard management practices such as irrigation and environmental conditions. For instance, the application of restricted or deficit irrigation results into a more continuous and thicker cuticular layer of the fruits compared to more cracked and thinner layer in overly irrigated fruit (Crisosto *et al.*, 1994; Peña *et al.*, 2013). This is because excess water causes increased turgidity of peel surface cells resulting in a stretched cuticle and decreased cuticle per unit surface area (Knoche *et al.*, 2004). Likewise, exposing the fruit to high relative humidity during storage can promote enlargement and micro-cracking of the fruit surfaces (Maguire *et al.*, 2000).

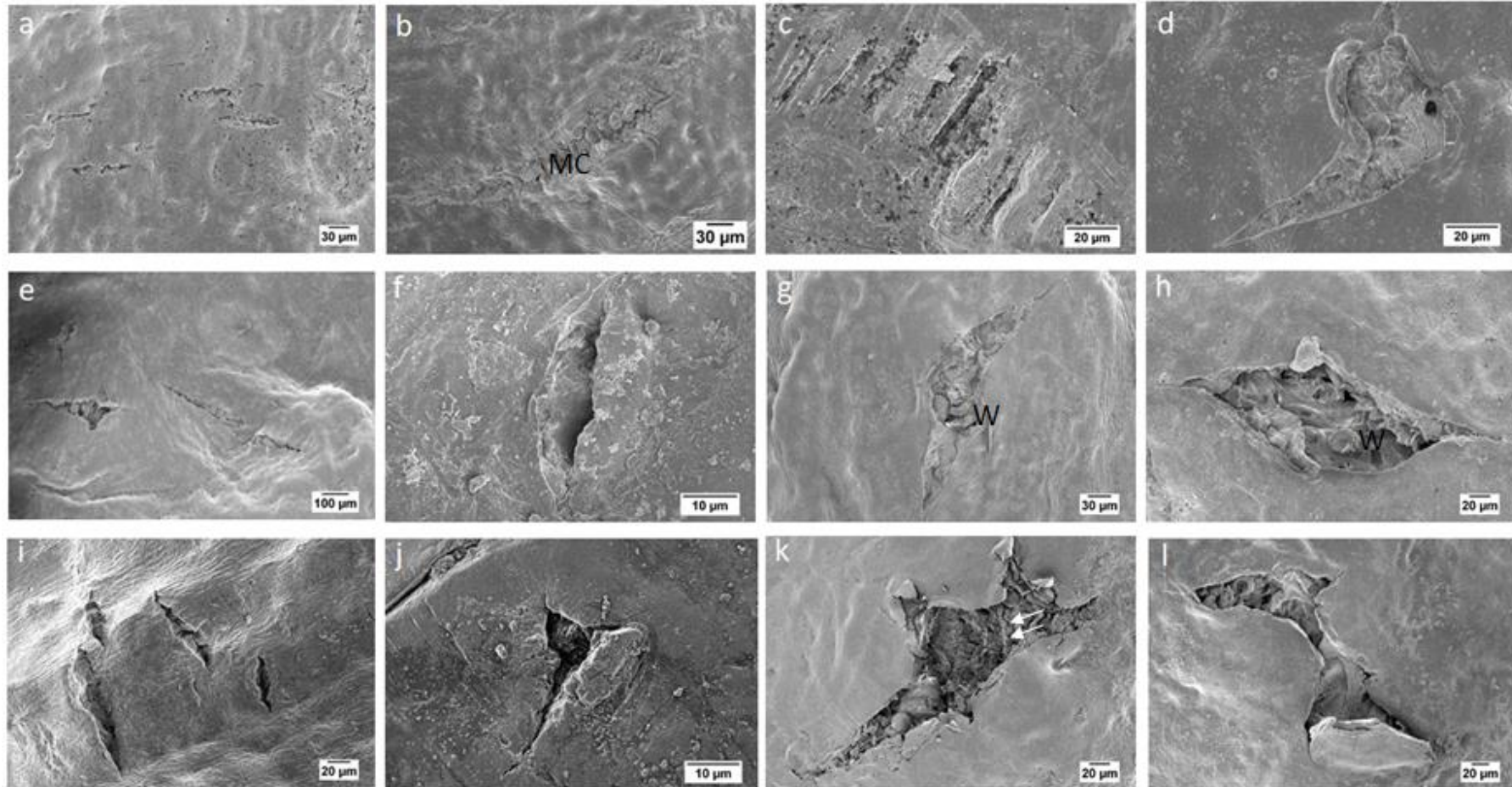


Figure 7.9 Changes in the micro-cracks structure on the surface of the pomegranate fruit peel (cv. Wonderful) during storage. The scanning electron microscope images were taken before fruit storage (**a-d**), after 42 d at 7 °C/90 % RH (**e-h**) and after additional eight days of shelf storage at 23 °C/58 % RH (**i-l**). Developing micro-crack covered by waxy material (MC), wax deposits inside cracks (W) and mycelium growth in the cracks (arrows).

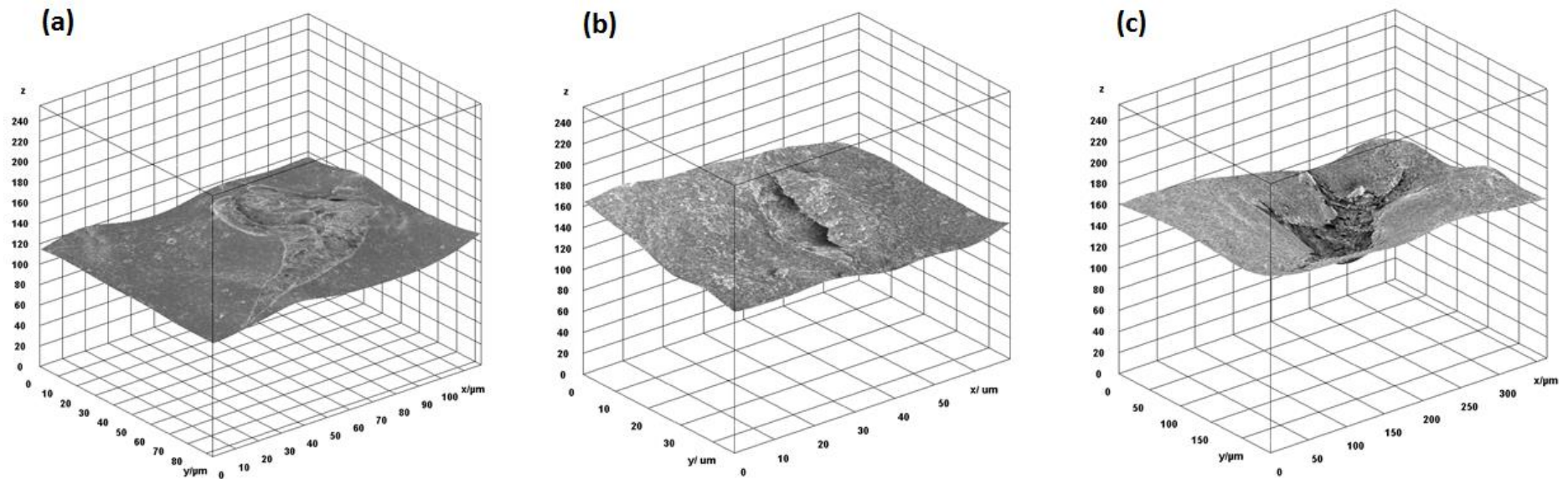


Figure 7.10 3D surface plots of cracks on the peel of pomegranate fruit (cv. Wonderful). Before fruit storage **(a)**, after 42 d at 7 °C/90 % RH **(b)** and after an additional eight days of shelf storage at 23 °C/58 % RH **(c)**. A noticeable depression around the crack at the end of shelf storage.

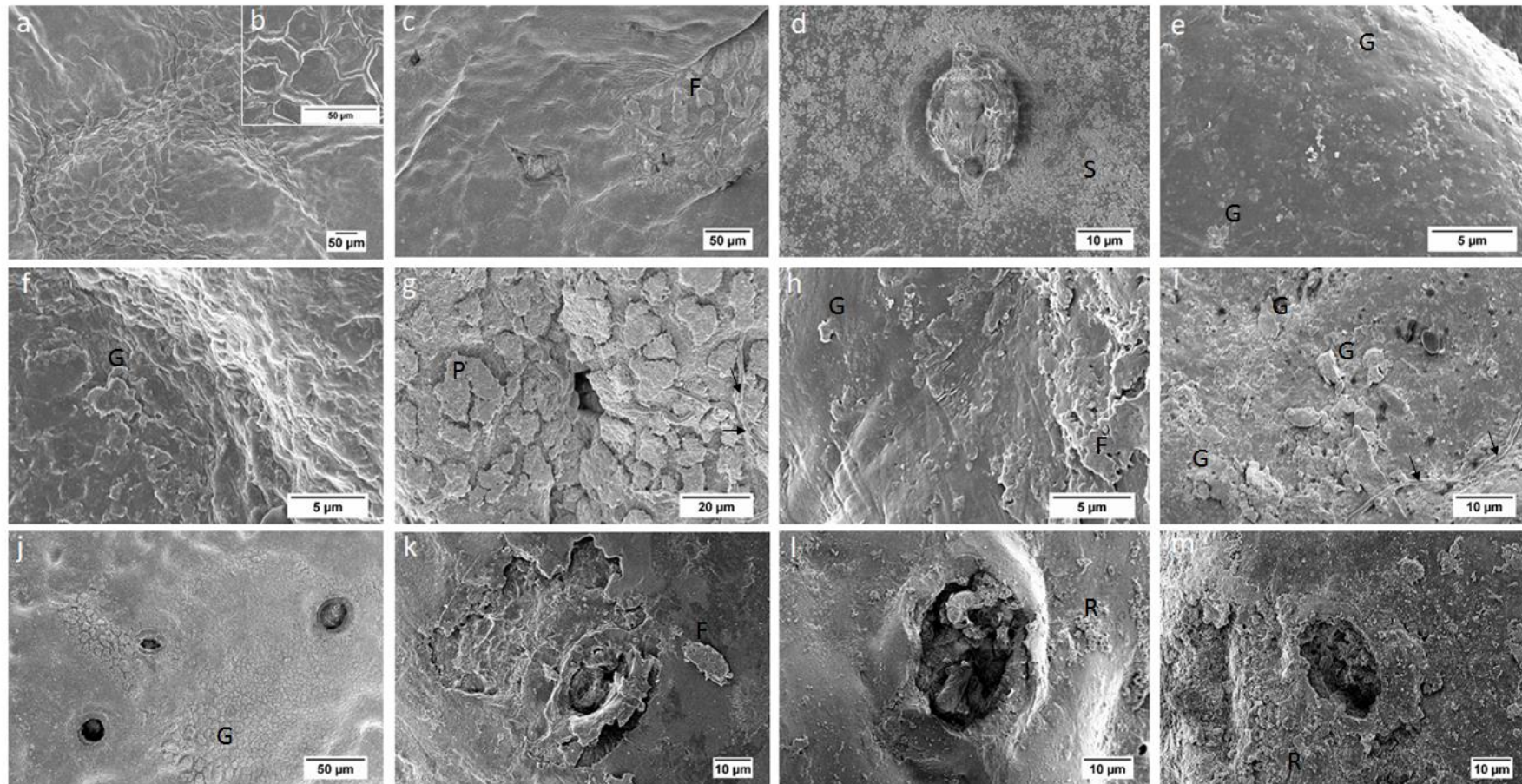


Figure 7.11 Wax layer patterns on the surface of the pomegranate fruit peel (cv. Wonderful) during storage. The scanning electron microscope images were taken before fruit storage (**a-i**), after 42 d at 7 °C/90 % RH (**j**) and after additional eight days of shelf storage at 23 °C/58 % RH (**k-m**). Hexagonal net pattern (**b**), granules (**G**), fragmentation (**F**), residues (**R**), stains (**S**), crystalline granules (**G**) and mycelial growth (**arrows**).

7.3.2.5 Surface wrinkles and wax patterns

SEM also revealed that the epidermal peel surfaces were more shrivelled by the end of the additional eight days of shelf storage than after 42 d of cold storage (**Figure 7.2**), as expected. **Figure 7.11a-m** shows the different patterns of wax covering at the different locations of the fruit during storage. The differences in the wax patterns on the fruit vary according to the stage of development, position on the fruit and prevailing environmental factors (Skene, 1963). Diversity of wax patterns included smooth, fragmented and lumpy (granulated) wax patterns. Similar, crystalline wax flocculents (lumps) and platelets (fragments) were observed in ‘Lobo’ and ‘Boskoop’ apple cultivars (Konarska, 2012). Before storage and at 42 d of cold storage more intact and un-broken waxy patterns were observed on the surfaces of the fruit as compared to more fragmented and lumped wax patterns at the end of the shelf storage. Contrary results have been reported in climacteric fruit, where a more smoothened and homogeneous wax covering was observed in apple fruit cultivars ‘Elstar’, ‘Jonagored’ and ‘Jonagold’ after 44 d of shelf storage at 20 °C (Veraverbeke *et al.*, 2001a). Wax deposits were also noticeable inside lenticels. Similarly, wax micro granules were observed in the stomata for plums (cv. Bluefre, Sweet Common Prune and President) (Konarska, 2015).

7.3.3 Identification of peel layers with CLSM

A distinction in the structure of the peel layers was observable under CLSM (**Figure 7.12a-c**). The pomegranate peel has lenticels and numerous micro-cracks on the surface of the peel and a distinctively bright waxy cuticle covering the outer epidermis (**Figure 7.12a**). The epidermis is made up of a single layer of cells and the lenticels extend through this layer. Underneath the epidermis are the parenchyma cells whose size increases and compactness decreases away from the epidermis (**Figure 7.12b**). Vascular bundles and sclerenchyma cells are observed between the parenchyma cells. The vascular bundles are more at the interface between the exocarp and mesocarp. These observations are similar to observation reported by Zhang *et al.* (2015). The pomegranate also has an inner epidermis that covers the arils (edible portion). The inner epidermis consists of uniformly sized, round-shaped and closely packed cells (**Figure 7.12c**).

The waxy cuticular layer is very distinctive and appears brighter than other layers. Part of the cuticle is observed on the surface (surface waxy cuticle) of the epidermis and the other part projects into the epidermis (intra-cellular cuticle). Similarly, in climacteric fruit such as the apples, the cuticle was observed to penetrate into the radial walls of the epidermal cells (Konarska, 2012). The cuticle consists of a matrix of the cutin polymer and wax in the form of

intra-cuticular wax and or epi-cuticular wax. These components of the cuticle tend to have varying fluorescent properties and can be identified with CLSM (**Figure 7.12b** and **7.13a-c**). The epicuticular wax was observed as a whitish layer of higher intensity as compared to the underlying cutin material. However, the two components tend to overlap into each other and are quite indistinguishable in some parts as revealed by the 3D projections of the surface layers (**Figure 7.14a-c**). The intra-cuticular wax is deposited in the cutin matrix while the epicuticular wax forms a wax film on top of the matrix and or distinctive crystalline structures (Schreiber and Riederer, 1996; Veraverbeke *et al.*, 2001b; Yeats and Rose, 2013). The epicuticular wax plays a big role in the glossiness of the product's surface and minimising of excessive moisture loss. Similarly, previous work on the anatomical structure of the pomegranate peel reports the presence of a cuticular layer covering the epidermis which consisted of one layer of cells (Yazici *et al.*, 2011).

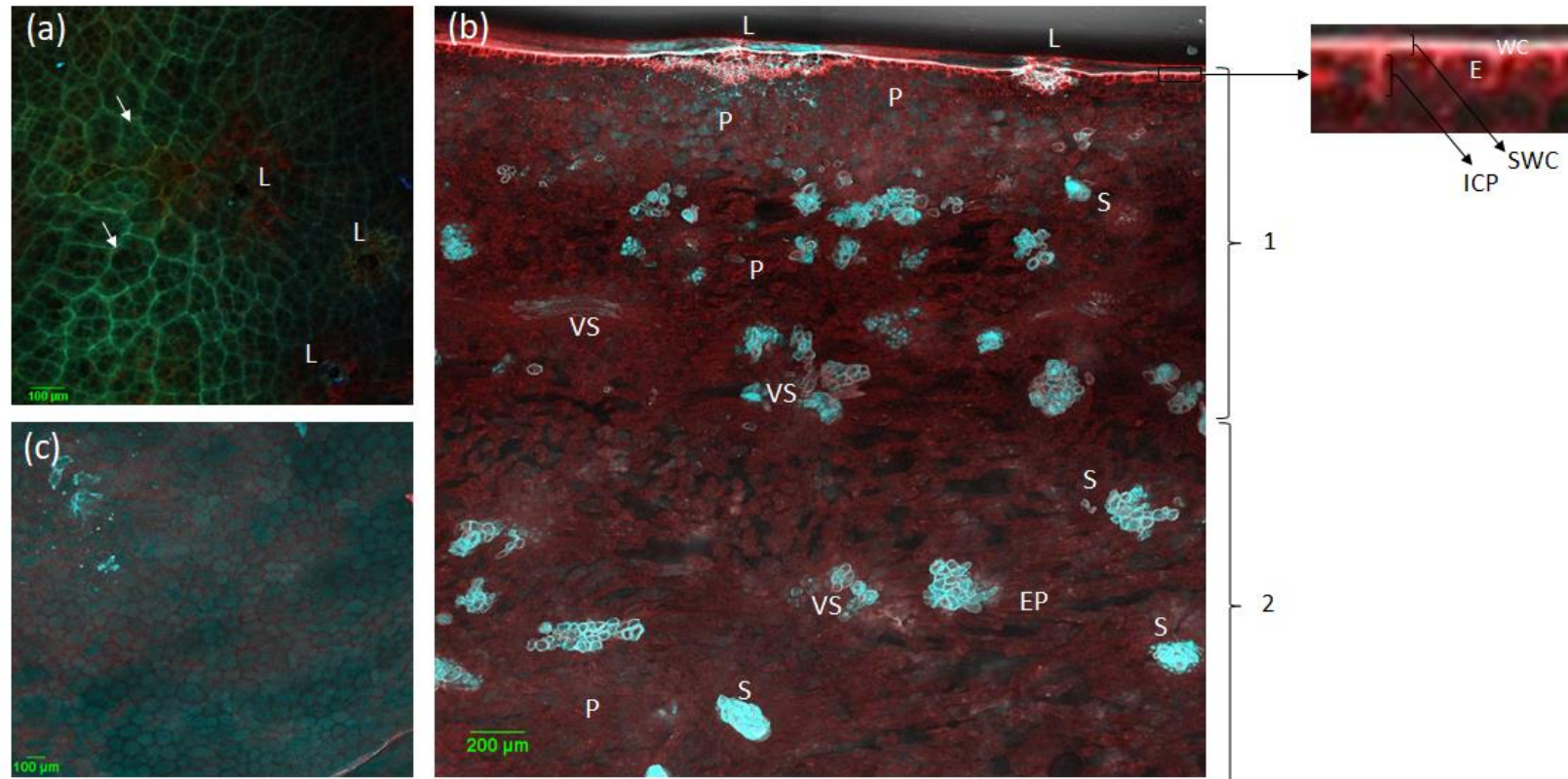


Figure 7.12 Peel layers of pomegranate fruit (cv. Wonderful) observed under confocal laser scanning microscopy. Outer most surface of the exocarp (a), transverse section through the peel (b) and the divider membrane (internal epidermal layer) that covers the arils (c), numerous micro-cracks covered with cuticle (arrows), lenticels (L), outer epidermis (E), waxy cuticle (WC), vascular bundles (VS), parenchyma cells (P), sclerenchyma cells (S), elongated parenchyma cells (EP), surface waxy cuticle (SWC), intracellular cuticular projection (ICP), exocarp/outer peel (1), mesocarp/inner peel (2). Image (a) was unstained while (b) and (c) were stained with Nile red dye.

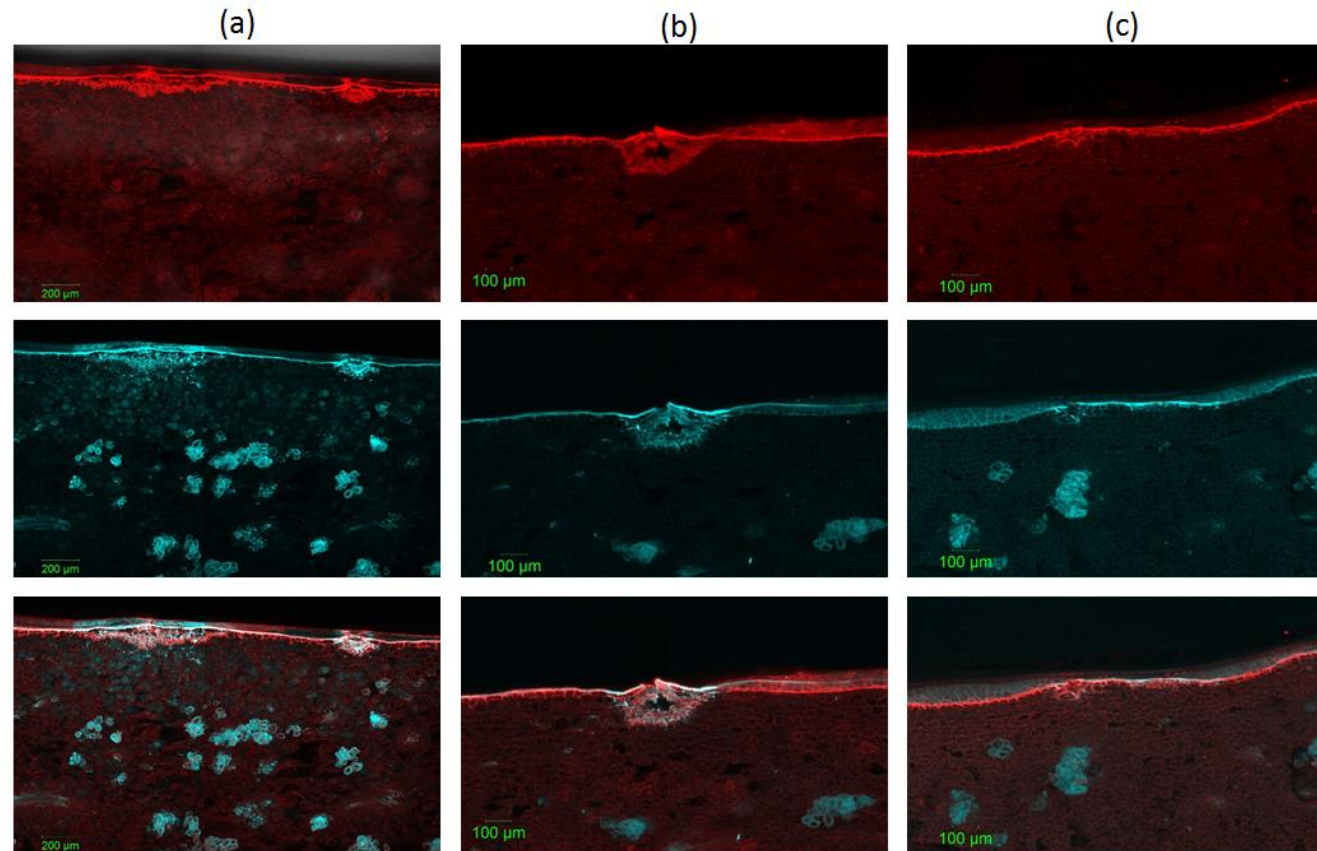


Figure 7.13 Sections through the peel of pomegranate fruit (cv. Wonderful). The freehand sections were stained with Nile red dye and viewed under a confocal laser scanning microscope. Before fruit storage **(a)**, after 42 d at 7 °C/90 % RH **(b)** and after an additional eight days of shelf storage at 23 °C/58 % RH **(c)**. Fluorescence of cell walls and cutin material (structural carbohydrate materials) (top images), the fluorescence of waxy and lipid-based materials (middle images) and the merged images (bottom images). The surface cutin material stands out in bright red and the waxy cuticle in bright cyan. A decrease in exocarp cell volume is noticeable with storage time.

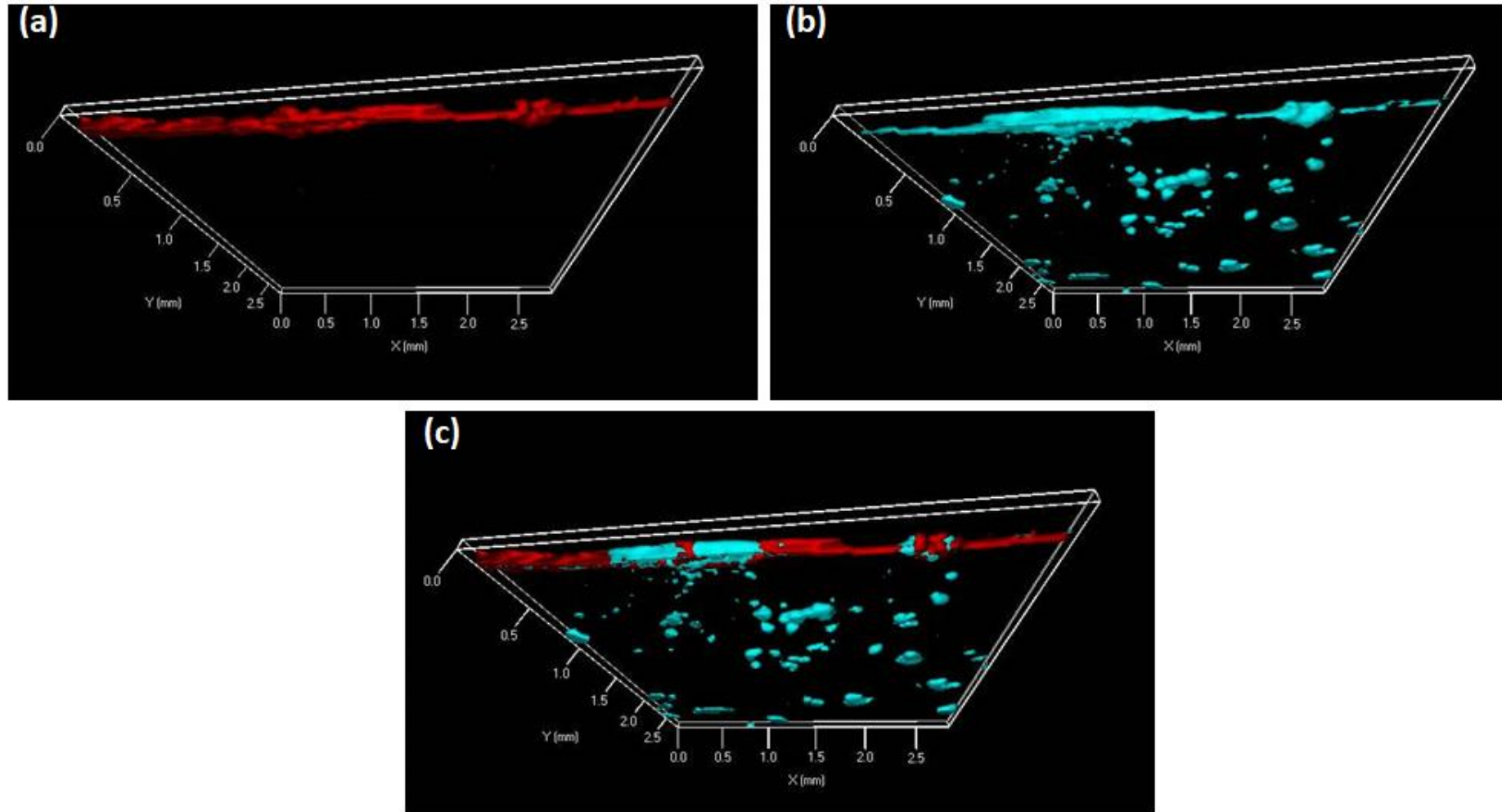


Figure 7.14 The 3D of the surface waxy cuticular layer for pomegranate peel scanned under a confocal laser scanning microscope. Fluorescence of the cutin material (a), the fluorescence of waxy and lipid based materials (b) and the merged image (c).

7.3.4 Changes in waxy cuticle thickness

Figure 7.15 shows a variation in the wax thickness across surface locations with storage duration. A generally decreasing wax thickness profile is portrayed during storage. Before storage, the surface wax thickness was highest at the bottom (23.5 μm), followed by the mid (20.9 μm) and least at the top (19.4 μm) locations on the surface of the fruit location. This partly explains why more lenticels count per unit area were observed at the top location, followed by the mid and then the bottom surface locations. However, by the end of the shelf storage period, the surface wax thickness was similar across all locations with an average of 7.3 μm . Generally, our results were relatively higher compared to a cuticular thickness of 19.32 and 13.64 μm reported in ‘Lobo’ and ‘Boskoop’ apple cultivars, respectively (Konarska, 2012). Furthermore, lower results of 4.6, 4.7 and 4.9 μm were reported in the ‘Bluefre’, Sweet Common Prune’ and ‘President’ plum cultivars, respectively (Konarska, 2015). The thinner cuticle in plum cultivars could partly explain the generally higher count of stomata of about eight compared to three lenticels per unit millimetre for pomegranate peel samples excised from the mid (equatorial) location on the fruit. On the other hand, the intra-layer wax projections were 25.3 μm deeper into the epidermis at the mid location as compared to 21.1 μm at the top and bottom locations before fruit storage and by the end of shelf storage intracellular wax thickness decreased to an average of 9.7 μm across all locations. The decrease in wax thickness during fruit storage could be attributed to re-distribution of wax on the fruit’s surface. Alternatively, it could be due to the falling off of the wax from peel surface during fruit storage as previously suggested by Zhang *et al.* (2015).

Figure 7.16a-f shows the general frequency distribution of surface wax and intra-layer wax thickness. Surface wax thickness varied over a wider range of 5-55 μm thickness before fruit storage compared to a reduced range of 5-30 μm at 42 d of cold storage and 5-15 μm at the end of shelf storage (**Figure 7.16a-c**). A similar variation pattern is observed for intra-layer wax thickness (**Figure 7.16d-f**). This implies that the wax thickness became more uniform in thickness with storage time and this can be attributed to horizontal re-distribution of wax on the surface and within the epidermal layer.

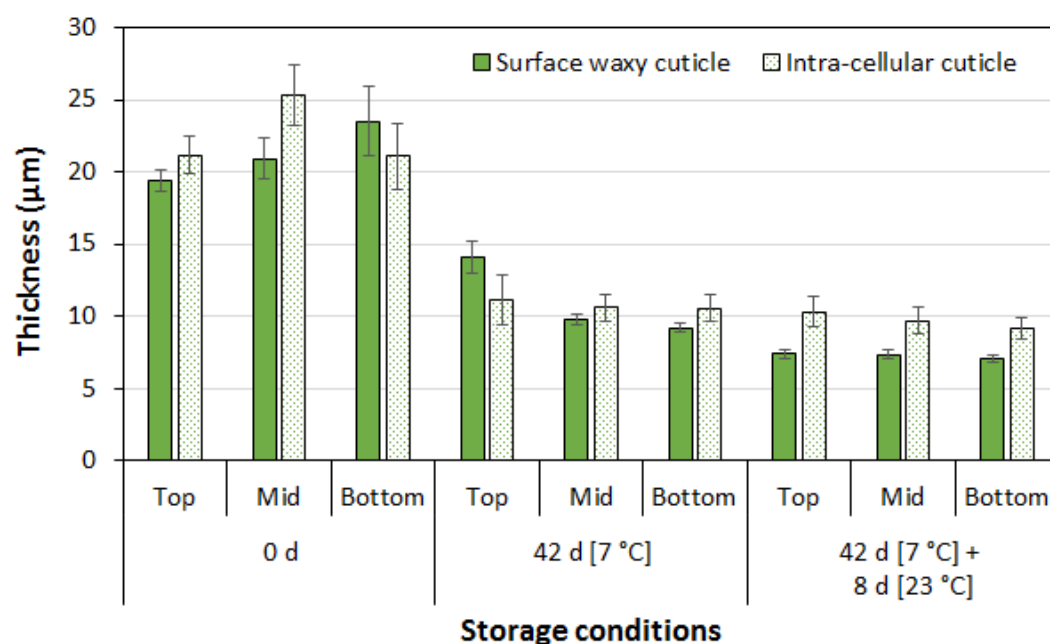


Figure 7.15 Variation in surface wax and intra-layer wax thickness with surface location and storage conditions of pomegranate fruit (cv. Wonderful).

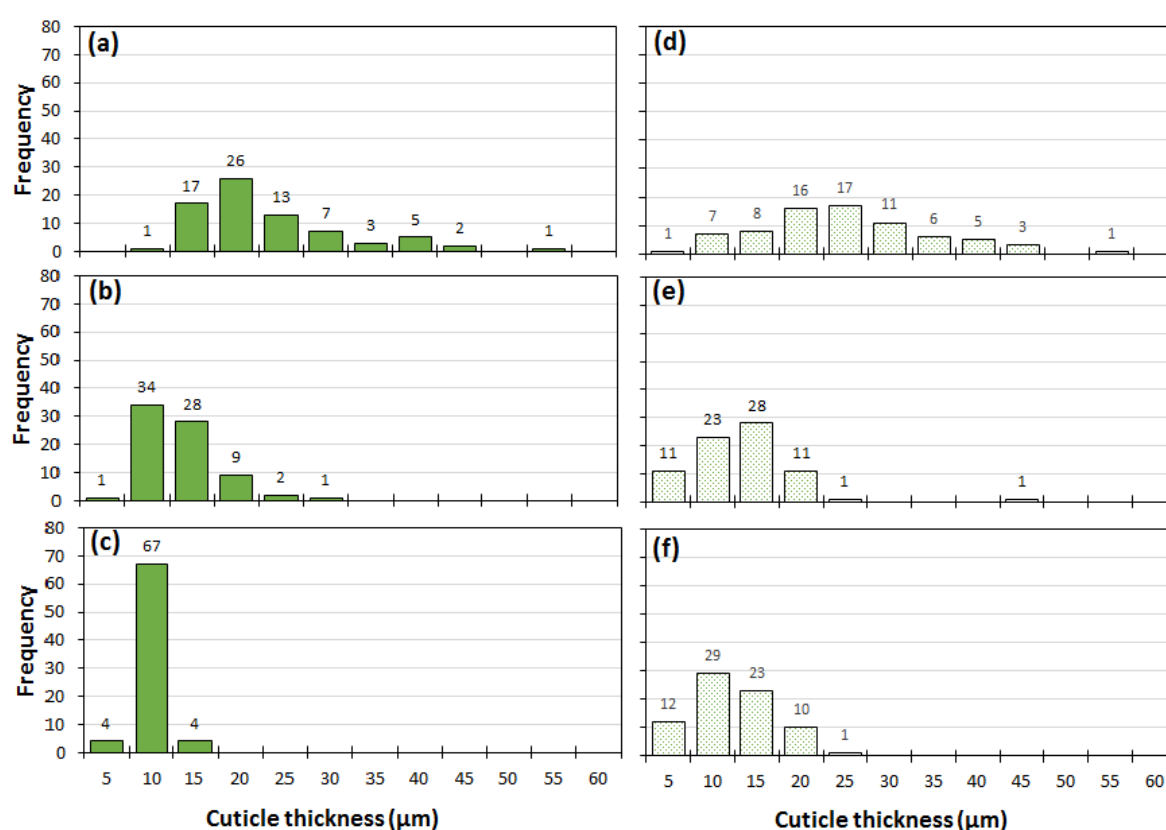


Figure 7.16 Frequency distribution of cuticle thickness during storage of pomegranate fruit (cv. Wonderful). Surface waxy cuticle (a-c) and intra-cellular wax (d-f) before storage (a and d), after 42 d at 7 °C/90 % RH (b and e) and after additional eight days of shelf storage at 23 °C/58 % RH (c and f).

7.3.5 Changes in macro peel fraction thickness

In our previous study, we established that moisture loss primarily and majorly comes from the peel (un-edible portion) while the arils retain their moisture content for pomegranate (cv. Wonderful) fruit stored for 42 d at 7 °C and 90 % RH and additional eight days of shelf storage at 23 °C and 58 % RH (**Chapter 4**). In this section, we present results on changes in the thickness of the peel and peel layers with respect to location on the fruit and storage conditions as summarised in **Figure 7.17a-e**. The thickness of the pomegranate peel varies greatly along the vertical plane depending on the location (top, mid and bottom) on the fruit and the point of measurement along the length of the peel due to alternating ‘crests or peaks’ between arils and ‘troughs’ directly below the arils. Overall peel thickness was generally lower at the top and mid than at the bottom locations on the fruit. Before storage, peel thickness was 5.4, 5.2 and 8.1 mm at the crest points and 3.5, 4.0 and 6.3 mm at the trough points, for top, mid and bottom locations, respectively (**Figure 7.17a-b**). A comparable 3-5 mm average peel thickness is reported in the equatorial region (mid location) of ‘Dahongpao’ cultivar (Zhang *et al.*, 2015).

By the end of shelf storage, peel thickness had decreased by 47.0, 42.0 and 39.5 % to a thickness of 2.9, 3.0 and 4.9 mm at the crest points and by 47.3, 49.2 and 43.1 % to 1.9, 2.1 and 3.6 mm at the trough points, respectively. This implies that the pomegranate fruit was more susceptible to moisture loss at its top and mid locations as compared to the bottom location. This is partly attributed to a higher count of surface openings, a larger fraction of open surface area and more circular and rounded openings that facilitate high rates of moisture loss as discussed in previous sections of this chapter. However, further knowledge of the under-surface microstructure of the peel is needed for a rich understanding of this phenomenon. In this current study, it was noticed that the decrease in overall peel thickness was more at the trough points directly below the arils than at the crest points in between the arils. This implies that the edible portion (arils) becomes more susceptible to impact damage due to decreased cushioning from the peel.

The mesocarp (inner peel) is the most abundant peel fraction by volume. The mesocarp contributes about 72.0, 70.1 and 96.0 % to the overall peel thickness at the crest points and 58.2, 59.5 and 87.9 % at the trough points for the top, mid and bottom locations, respectively and the rest was due to the exocarp (outer peel) fraction. Its large volume in addition to its spongy nature and positioning, makes it suitable as a shock absorber, protecting the arils against impact damage. The top, mid and bottom locations had a mesocarp thickness of 3.8, 3.5 and

5.3 mm at the crest points and 2.0, 2.4 and 4.1 mm at the trough points before fruit storage, respectively (**Figure 7.17c-d**). At the end of 42 d cold storage, the mesocarp thickness had decreased by 20.1, 24.4 and 9.4 % at the crest points and by 27.5, 25.8 and 15.6 % at the trough points, respectively. The additional eight days of shelf storage resulted in extra 19.4 and 21.8 % general decrease in the mesocarp thickness at the crest and trough points, respectively. On the other hand, the exocarp thickness generally decreased by 34.0, 27.2 and 22.0 % at the top, mid and bottom locations by the end of cold storage and an extra 25.0 % decrease by the end of shelf storage (**Figure 7.17e**). Therefore, the exocarp peel layer was more collapsed during fruit storage than the mesocarp layer in the range of the tested conditions. The collapse in exocarp cell volume is evidenced in **Figure 7.13** above. A progressive decrease in the white membrane thickness was also observed (**Figure 7.18**) from 0.24 to 0.08 mm by the end of the study. White membrane thickness decreased by 20 % and 67.9 % at 42 d of cold and after eight additional days of shelf storage, respectively. Therefore, all the fractions of the un-edible portion of pomegranate fruit are observed to decrease in thickness and this is attributed to moisture movement from the inside of the fruit to the surrounding environment.

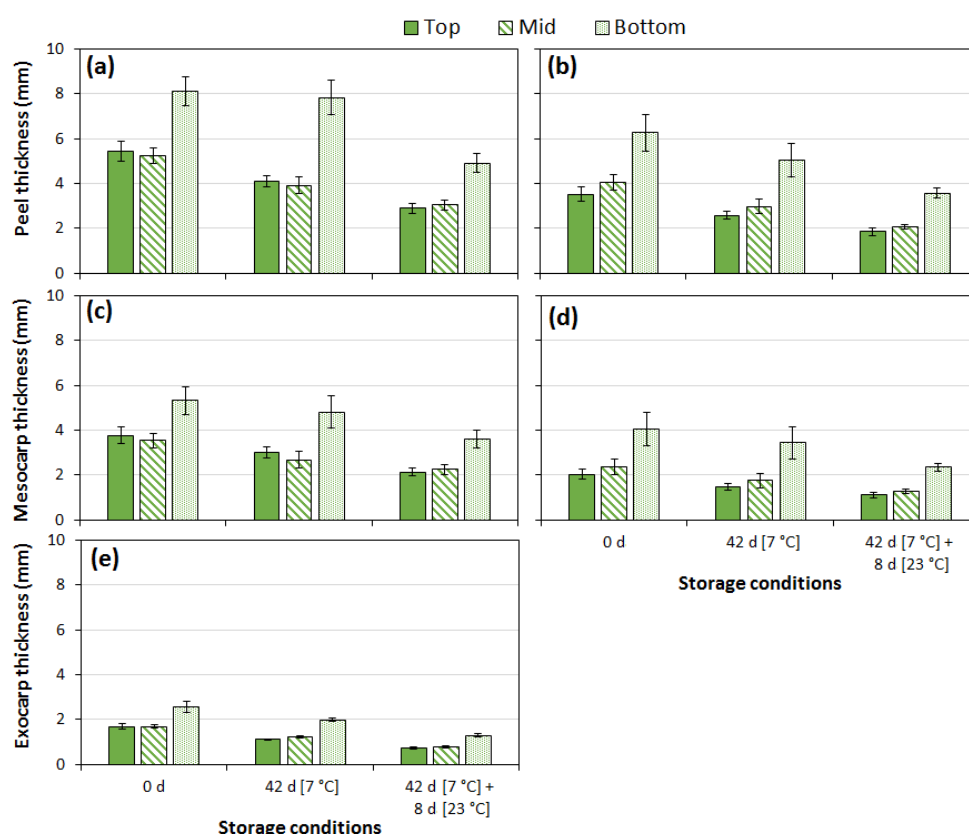


Figure 7.17 Peel thickness of pomegranate fruit (cv. Wonderful). Overall peel thickness at the crest (a) and trough (b) points, mesocarp thickness at the crest (c) and trough (d) points and average exocarp thickness (e).

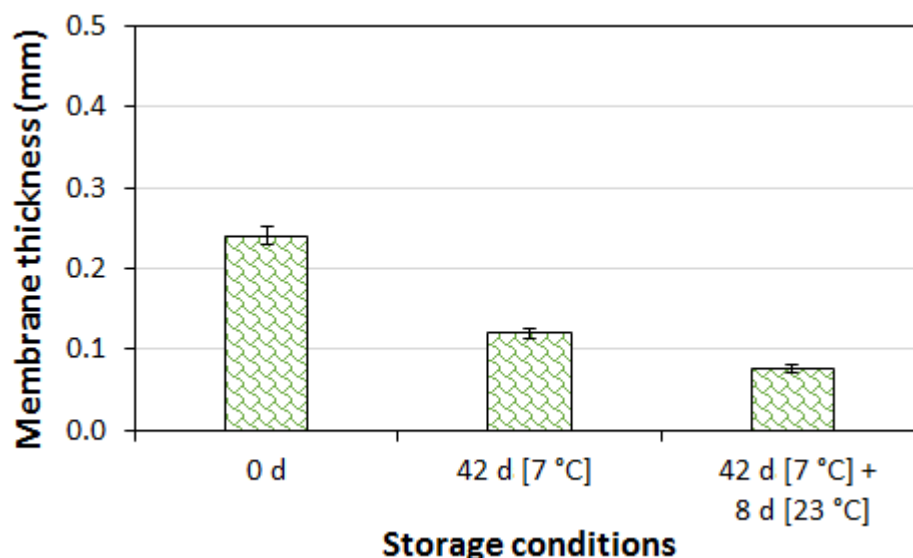


Figure 7.18 Divider membrane (inner epidermis) thickness of pomegranate fruit (cv. Wonderful).

7.4 Conclusion

The examination of the pomegranate peel surface with SEM identified several features including surface openings such as lenticels of various shapes and micro-cracks and waxy cuticle fragmentations. The lenticels were commonly observed as either round, oval, lens-shaped or stomata-like, with the majority being stomata-like in appearance. A distinctively bright waxy cuticle was identified on the surface of the peel using CLSM. A great variation in the overall peel thickness was noticeable with respect to location on the fruit, point of measurement and storage conditions.

A higher count of lenticels, larger lenticel size, greater porosity, circularity and roundness of the individual lenticels, generally low peel thickness suggest that the pomegranate fruit was more susceptible to moisture loss at its top and mid locations as compared to the bottom location. Therefore, these findings suggest that weight loss control techniques such as surface waxing coating can be optimised through the strategic application on the different locations of fruit without necessarily compromising on the aerobic respiration process of the fruit.

Furthermore, a decreasing waxy cuticle thickness profile, increased fragmentation of waxy cuticle, widening and deepening of micro-cracks, noticeable shrivelling and the general decrease in peel thickness were observed during fruit storage. This suggests that the general appearance of the fruit and the integrity of the peel were greatly compromised by end of shelf

storage. Therefore, the use of surface protective coating and waxing should be considered in minimising fruit weight loss and retention of the general quality. Generally, this study partly established the susceptibility of pomegranate fruit to excessive moisture loss because of the various surface openings (lenticels and micro-cracks) on the surface. However, the under-skin microstructure of the thick rind remains largely anonymous and therefore needs to be investigated.

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CHAPTER 8

ANALYSING THE 3D MICROSTRUCTURAL PORE SPACE OF POMEGRANATE PEEL FRACTIONS WITH FRUIT WEIGHT LOSS USING X-RAY MICRO-CT

With regard to Chapter 8, pages 210–231, the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Research, data collection and analysis, and writing of chapter	80

The following co-authors have contributed to Chapter 8, pages 210–231:

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Declaration with signature in possession of candidate and supervisor Signature of candidate	26/02/2020 Date
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Declaration by co-authors

The undersigned hereby confirm that:

1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 8, pages 210–231,
2. no other authors contributed to Chapter 8, pages 210–231 besides those specified above, and
3. potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 8, pages 210–231 of this dissertation.

Signature	Institutional affiliation	Date
Declaration with signature in possession of candidate and supervisor	Department of Horticultural Sciences, Stellenbosch University	26/02/2020
Declaration with signature in possession of candidate and supervisor	Department of Horticultural Sciences, Stellenbosch University	26/02/2020

Chapter 8

8 Analysing changes in the 3D microstructural pore space of pomegranate peel fractions with fruit weight loss using X-ray micro-CT

Abstract

The transport of water and gases across the fruit peel is greatly influenced by its microstructural characteristics which change with fruit physiology during storage. There is limited information on the structure of fruit with thick peel (rind), including the pomegranate (*Punica grantum* L). This study reports the first scientific results on the changes in the 3D microstructural pore space of pomegranate peel due to moisture loss, using X-ray micro-computed tomography. Cuboid samples were excised from the top (near the calyx), middle (equatorial region) and bottom (near the pedicel) locations on randomly selected fruit before storage, after 42 d at 7 °C and 90 % RH and after additional 8 d at 23 °C and 58 % RH. Pore space ratio and pore characteristics such as distribution, sphericity, compactness and surface area projection within the exocarp (outer peel) and mesocarp (inner peel) fractions were analysed. The results showed observable variations of pore space fraction with sample location on fruit, peel fractions and storage duration. Pore space was highest in the top (26.1 %), followed by middle (19.3 %) and then the bottom (15.8 %), especially in the exocarp. This increased with storage duration in the exocarp compared to a decrease in the mesocarp. The pores were more projected in the XZ-plane, thereby facilitating the vertical flow of fluids from the inside to the outer surface. This research is a fundamental step towards the understanding of the weight loss mechanisms in pomegranate fruit and the findings are important in modelling of water transport and design of strategic weight loss control techniques.

8.1 Introduction

Pomegranate (*Punica grantum* L) fruit is valued for its innumerable health benefits and researchers have linked its potent pharmacological activities to the several groups of phytochemicals in the arils (edible portion) and in the peel of the fruit (Viuda-Martos *et al.*, 2010; Fawole *et al.*, 2012a,b; Mphahlele *et al.*, 2016). The arils are segmented and separated by membranous walls and the whole fruit interior is dressed over with a leathery outer coat

(epicarp/exocarp). The revived interest in this ancient fruit has resulted in increased production, consumption and intensified research owing to its health and nutritional benefits (Aviram *et al.*, 2008; Guo *et al.*, 2008; Opara *et al.*, 2009; Arendse *et al.*, 2014). However, the fruit is classified as highly perishable (Barman *et al.*, 2011) despite its relatively lower respiration rate during postharvest storage (Caleb *et al.*, 2012). The fruit is specifically prone to moisture loss owing to the relatively numerous micro openings on the peel, irrespective of its thick rind and tough leathery outer skin (Opara *et al.*, 2010; Fawole and Opara, 2013).

The transport of water and the intake and evolution of gases across the fruit peel is greatly influenced by the microstructural characteristics which change with the physiology of the fruit during storage. The demand to understand the general micro-structural space of fruit with thick rind such as pomegranates is on the rise. This has been made possible with technologies such as X-ray micro-computed tomography (X-ray μ CT) with diverse applications in the food industry (Schoeman *et al.*, 2016). This imaging technique creates contrast among fruit structures based on their ability to attenuate X-rays owing to differences in mass densities of the fruit material, with pore space visualised as regions with low intensities. Magwaza and Opara (2014) investigated the potential of X-ray CT to identify and quantify the internal structures of pomegranate fruit (cv. Shani-Yonay). Likewise, Arendse *et al.* (2016) successfully and non-destructively quantified the internal and external structures of pomegranate fruit (cv. Wonderful) using X-ray μ CT. Similar studies were conducted on other pomegranate cultivars (cvs. Rabab Malas and Rabab Torsh) (Salmanizadeh *et al.*, 2015). In other types of fruit, high resolution computed tomography has been applied to critically investigate the pore space within the different fruit tissues. Mendoza *et al.* (2007) used X-ray μ CT to visualise and characterise the 3D microstructure and pore space network of apple tissue. Herremans *et al.* (2015) characterised the 3D microstructure of apple (cvs. Jonagold, Kanzi and Braeburn) and pear (cv. Conference) parenchyma tissue, considering individual cells and intercellular spaces. Cantre *et al.* (2014) investigated changes in the 3D microstructure of mango (cv. Carabao) during ripening. Similar studies have been conducted on various plant-based tissues such as in kiwi fruit (Cantre *et al.*, 2014a), frozen apple (Vicent *et al.*, 2017), fried potato chips (Alam and Takhar, 2016), eggplant and turnip (Nugraha *et al.*, 2019) and cucumber (Kuroki *et al.*, 2004).

Simulation of viscous transportation of water was for the first time recently attempted on the microstructure of pomegranate peel using X-ray μ CT (Ambaw *et al.*, 2018). However, very little information is known about the changes that occur in the peel microstructure of fruit

with thick rind during postharvest storage. The aim of this study was to investigate changes in the 3D microstructural pore space of pomegranate peel as a result of fruit weight loss. This was achieved by using X-ray CT to specifically characterise the pore space variation with respect to location (top, middle and bottom) on the fruit, peel (outer and inner peel) fractions and storage duration. This research is a fundamental step towards the understanding of the weight loss mechanisms in pomegranate fruit. The findings of this research can be applied in the modelling of water transport and design of strategic weight loss control techniques.

8.2 Materials and methods

8.2.1 Fruit acquisition

Pomegranate fruit (cv. Wonderful) at acceptable commercial maturity were carefully and individually harvested by hand from a commercial orchard situated in Porterville, Wellington (33° 38' S, 19° 00' E), Western Cape Province, South Africa. Fruit were transported in ventilated plastic trays cushioned with paper pads to the postharvest research laboratory, Stellenbosch University. Sorting of fruit was carried out to ensure size uniformity and that fruit were free from surface defects such as cracks. Fruit were packed in dozens inside single layer display type paper cartons, cushioned with paper trays at the bottom.

8.2.2 Experimental design

A total of 36 fruit were distributed into two batches; 12 fruit were used in the weight loss study while the remaining 24 fruit were allocated for the peel pore space analysis experiment with X-ray Nano computed tomography scanning system. Six fruit were used in the scanning process before storage and the remaining fruit were stored in a preconditioned cold room at 7 ± 1 °C and 90 ± 5 % RH (relative humidity) for 42 d, mimicking the sea freight duration from South Africa to Europe across the Atlantic Ocean. Six more fruit were sampled immediately after cold storage and after the additional eight days of shelf storage at 23 ± 3 °C and 58 ± 5 % RH.

8.2.3 Weight loss monitoring

A set of 12 randomly selected fruit were labelled and monitored for a cumulative change in weight. The same fruit were weighed individually at 0 d (before storage) and at intervals of seven days throughout the 42 d of cold storage and thereafter at intervals of two days for the additional 8 d storage at shelf conditions, using an electronic scientific scale (Mettler Toledo,

model ML3002E, Switzerland, 0.0001 g accuracy). Cumulative weight loss was then calculated and expressed in percentage using **Equation 8.1**.

$$WL = 100/W_i \times (W_i - W_t) \quad (8.1)$$

Where WL is fruit weight loss (%), W_i is the initial weight (g) of fruit and W_t is the weight (g) of the same fruit at a specific sampling time (t).

8.2.4 Sample preparation before scanning

The cuboid shaped samples of dimensions $2 \times 2 \times 5$ mm each, were excised from the top (near the calyx), mid (equatorial region) and bottom (near the pedicel) locations on the individual fruit using sharp blades (**Figure 8.1**). Each of the dissected specimens was further sliced in to two separate parts: the top part (containing the reddish epidermis part of the peel) and the underneath white fleshy part of the peel (mesocarp). Hence, the scanning was done for the two layers separately. A specimen was immediately wrapped in parafilm to prevent water loss and then fixed to a specimen holder during scanning.

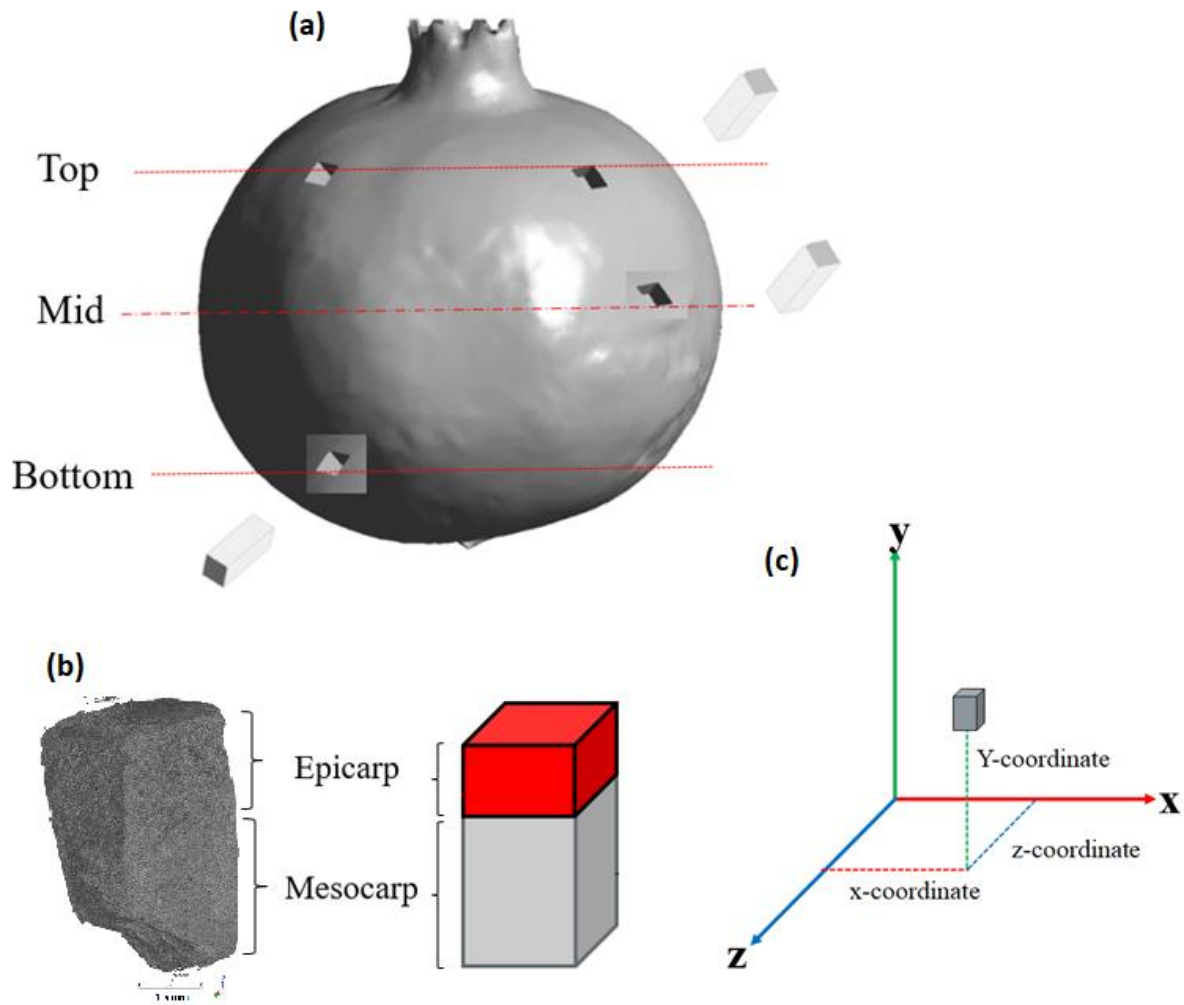


Figure 8.1 Schematic illustration of the locations on the fruit (Top, Mid and Bottom) from where the samples were excerpted (a), the distinct fractions of the peel (exocarp: outer peel and mesocarp: inner peel) (b) and sample registration along each of the axes of the scene coordinate system (yz-, xz- and xy- planes) (c).

8.2.5 X-ray micro-computed tomography imaging

Scanning was performed at the Stellenbosch CT scanner facility (du Plessis *et al.*, 2016b) with a commercial X-ray nano computed tomography system (Nanotom S, General Electric Sensing and Inspection Technologies/Phoenix X-ray, Wunstorf, Germany). The pomegranate peel sample was scanned at a voxel resolution of 3 μm , following optimised parameters according to the guidelines in du Plessis *et al.* (2017). X-ray radiations were generated from a source voltage of 60 kV with an electron current set at 240 μA . A total of 2400 images were recorded in one 360° rotation and with fast scan option activated, a scanning time of 40 minutes per sample was used. Reconstruction was applied to obtain a stack of images (2300 \times 2300 pixels)

using a system-supplied reconstruction software (Datos|x@2.1, General Electric Sensing & Inspection Technologies GmbH, Phoenix, Wunstorf, Germany).

8.2.6 Image processing

The 3D image visualisation and analysis were performed with Volume Graphics VGStudioMax 3.2. The Adaptive Gauss filter was applied to smoothen the image data by removing noise, followed by the selection of a $1.5 \times 1.5 \times 1.5$ mm region of interest (ROI). The advanced surface determination was carried out by applying a surface fit function to precisely define the edge between material and pore space, based on the threshold value between the peak of material and space in the data histogram. This process involves a global threshold, locally optimised at every point to minimise human bias and limit the effect of brightness changes across the images. du Plessis *et al.* (2016) previously applied this method with success in porosity analysis of medical implants.

8.2.7 Pore space analysis

An automated defect analysis was carried out using the porosity/inclusion analysis module of the Volume Graphics VGStudioMax 3.2. This analysis was carried out on both the exocarp (outer peel) and mesocarp (inner peel) fractions. Information on each individual pore space was generated including volume, diameter, projected surface area, sphericity and compactness. The total volume, material volume and pore space volume in each region of interest (ROI) were automatically calculated using the volume analyser tool. Porosity (pore space ratio) was automatically calculated as the ratio of pore space to the total space in the selected ROI using **Equation 8.2**.

$$Porosity (\%) = 100 \times \frac{V_p}{V_t} \quad (8.2)$$

Where V_p is the pore space volume of (mm^3) in a selected ROI and V_t is total space volume (mm^3) of the ROI.

Pore sphericity and compactness are shape factors indicating the extent to which an individual 3D pore space compares with an equivalent perfect sphere. Sphericity and compactness were automatically calculated using **Equation 8.3** and **8.4**, respectively.

$$Sphericity = \frac{A_s}{A_{pi}} \quad (8.3)$$

$$Compactness = \frac{V_{pi}}{V_s} \quad (8.4)$$

Where A_{pi} (mm^2) is surface area of an individual pore and A_s (mm^2) is surface area of a sphere with equivalent volume as the pore. Likewise, V_{pi} is volume of an individual pore (mm^3) and V_s is the volume of the circumscribed equivalent sphere (mm^3).

8.3 Results and discussions

8.3.1 Fruit weight loss

A cumulative increase in fruit weight loss with storage duration was observed (**Figure 8.2**). The pomegranate fruit lost about 8.2 % of weight by the end of the 42 d of cold storage at 7 °C and 90 % RH. A tremendous increase in weight loss rate is observed when the fruit were transferred to shelf conditions resulting into an extra 8.7 % (total of 16.9 %) weight loss by the end of the eight days storage at 23 °C. Our results were quite comparable with the 5.1 % weight loss in ‘Wonderful’ cultivar after 42 d storage at 5 °C and 90 % RH (Lufu *et al.*, 2018) and the 9.8 % weight loss in ‘Primosole’ after 42 d storage at 8 °C and 85-90 % RH plus an additional 7 d shelf storage at 20 °C and 65-70 % RH (D’Aquino *et al.*, 2010). The slight differences in the above studies can be attributed to variations in storage conditions (temperature and RH) and cultivar differences. The weight loss profile of the fruit caused observable changes in the pore space microstructure of the fruit peel.

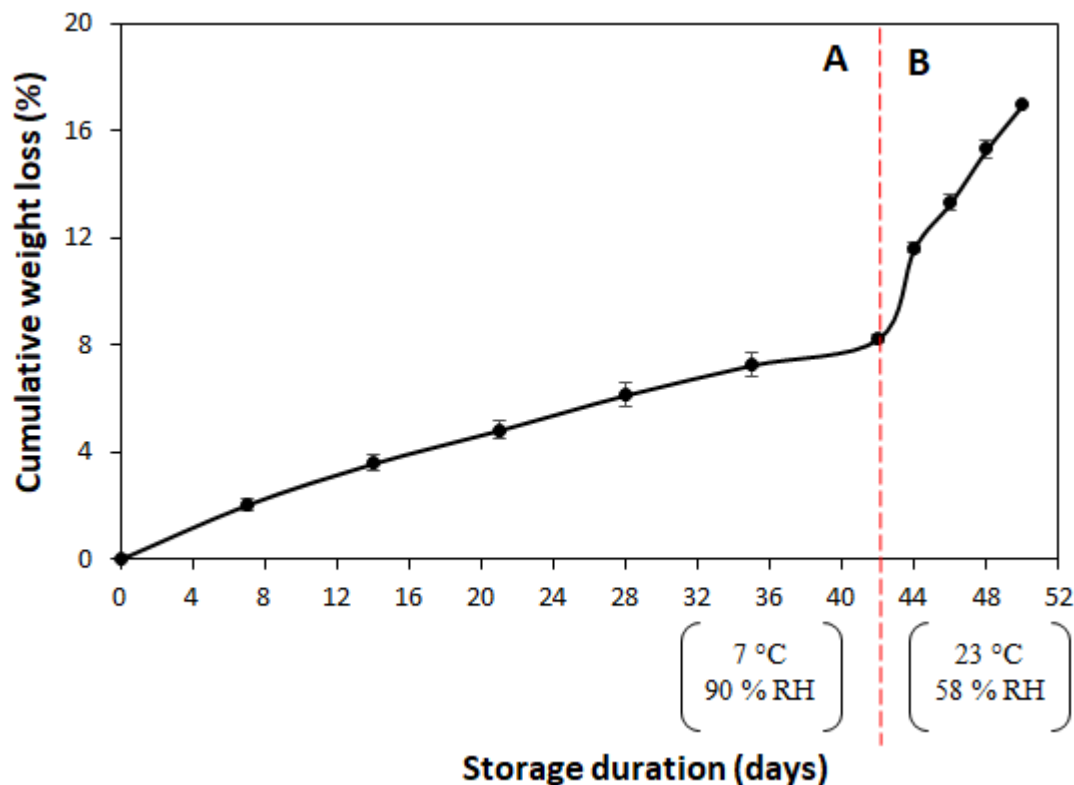


Figure 8.2 Cumulative weight loss profile of the same pomegranate fruit (cv. Wonderful) during cold storage at 7 °C/90 % RH (A) and subsequent shelf storage at 23 °C/58 % RH (B). The means of 12 fruit are presented and the vertical lines are error bars at $P \leq 0.05$.

8.3.2 Pore space in peel fractions

Generally, there was more pore space observed in the exocarp fraction (15.8 – 26.1 %) of the peel than in the mesocarp fraction (3.7 – 10.6 %) before fruit storage (**Figure 8.3**). This could be attributed to differences in cell arrangement and material organisation in the different tissues. For example, anisotropy is reported in apple parenchyma tissues where cells vary in shape and intercellular spaces with more spherical cells near the surface and elongated cells towards the fruit centre (Khan and Vincent, 1990). The authors observed intercellular spaces of 0.1 – 0.2 mm diameter near the surface compared to 0.05 – 0.1 mm towards the fruit centre. Furthermore, the degree of cellular organisation increased from random outer cells to radially oriented inner cells. In the current study, the mesocarp peel fraction has more tightly compacted cell material and smaller sized pores compared to loosely packed cells and larger open pores in the exocarp tissue (**Figure 8.4** and **8.5**). On the contrary, Zhang *et al.* (2015) report that the mesocarp consists of large parenchyma cells arrayed in a loose arrangement as compared to the smaller parenchyma cells of the exocarp for cultivar ‘Dahongpao’ of pomegranate. However, in this current study, larger patches of void space were common within the exocarp

and this coincided with frequent strands of vascular bundles than in the mesocarp. It is important to note that cultivar differences have a significant influence on the porosity values. Quite like our results, the intercellular space of 1 – 25 % was reported in apples and pear (Verboven *et al.*, 2008) however, pore space fraction can go as high as 28 – 32 % depending on the fruit cultivar (Mebatsion *et al.*, 2006).

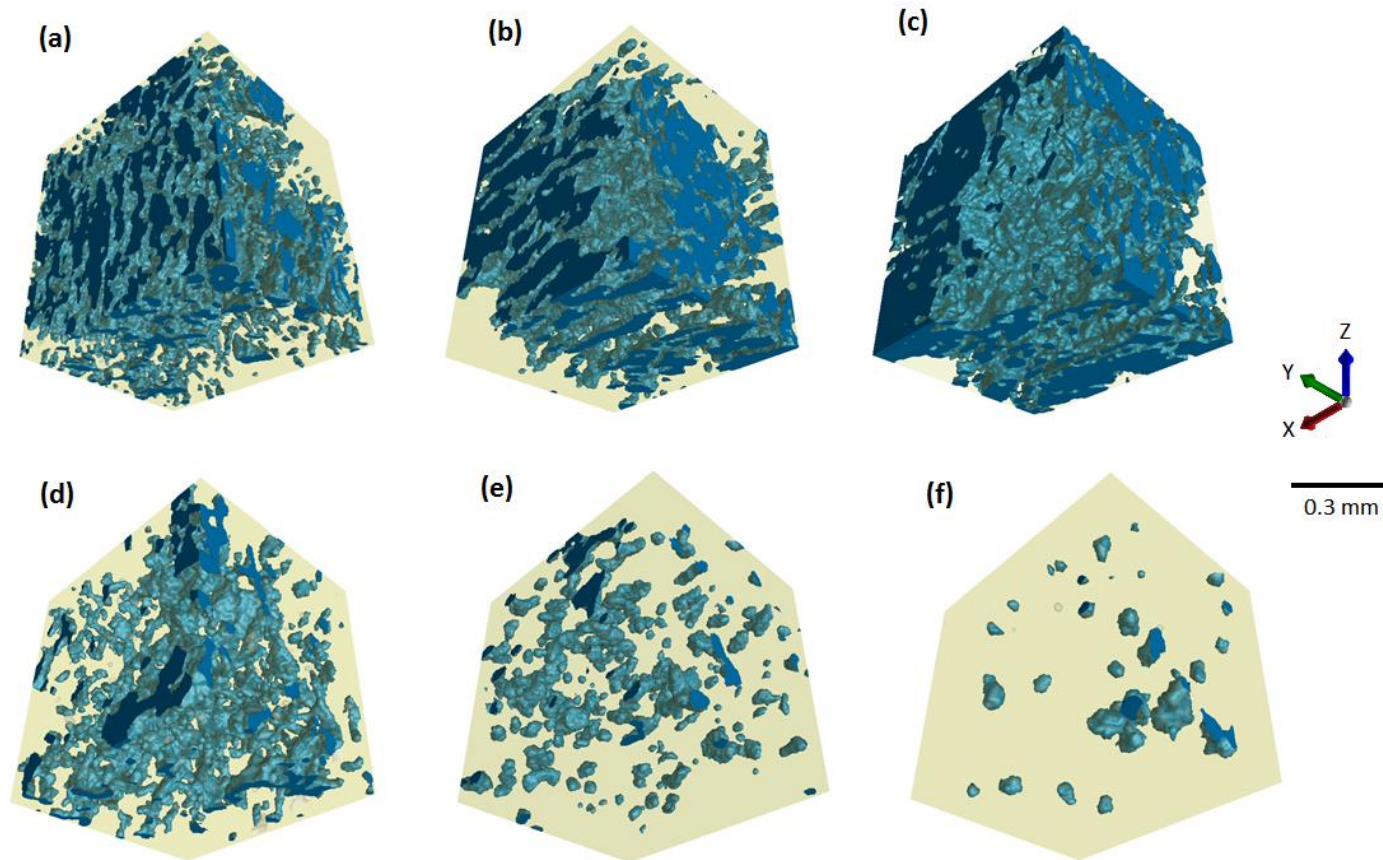


Figure 8.3 Changes in the 3D microstructural pore space of pomegranate peel fractions with fruit storage time. Structure of exocarp (outer peel) and mesocarp (inner peel) before storage (a and d), after 42 d at 7 °C/90 % RH (b and e) and after additional 8 d at 23 °C/58 % RH (c and f), respectively. The yellow regions represent peel material while the blue regions represent pore space. All samples were obtained from the top location on the fruit.

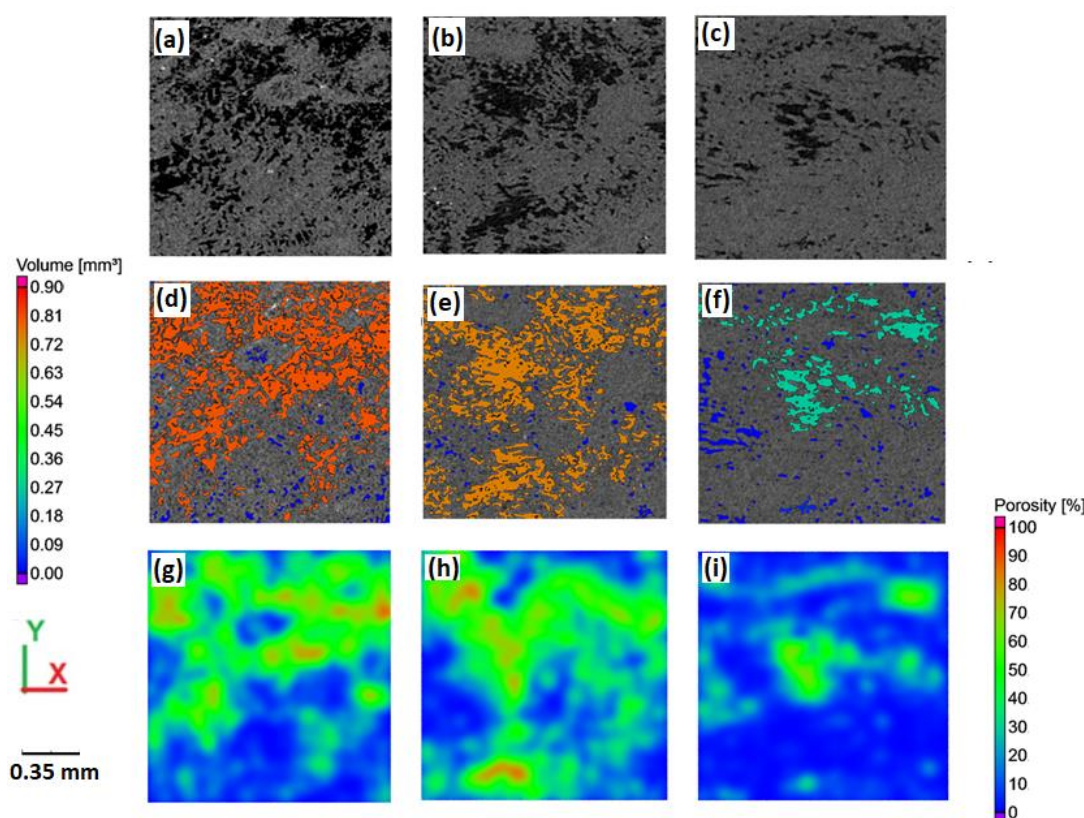


Figure 8.4 Variation of the 2D pore space distribution in the exocarp (outer peel) fraction with location on the pomegranate (cv. Wonderful) fruit compared at 0 d (before storage). Representative μ CT images from the Top (a), middle (b) and bottom (c) locations on the fruit, their analysed pore space volume (d), (e) and (f) and porosity distributions (g), (h) and (i), respectively.

8.3.3 Pore space distribution with location on fruit

Generally, there was an observable variation of pore space with position on the fruit, especially in the exocarp fraction of the peel. In the exocarp fraction (outer peel), pore space was highest in the top position (near the calyx), followed by the mid position (equatorial region) and least in the bottom position (near the pedicel) irrespective of the storage conditions (**Figure 8.4**). These findings are in agreement with results from the previous study (**Chapter 6**) where a relatively higher water permeability and diffusivity was observed in the top than in the mid and bottom located samples. This is probably due to natural mechanisms established during tissue development and fruit growth against excessive transpiration, where regions of the leaves and fruit that are more exposed to sunshine intensity tend to have more compacted cells, fewer surface openings and thicker waxy cuticles (Rehman *et al.*, 2015). The pomegranate fruit are orientated on the tree in a way that the top (calyx side) faces down towards the ground while

the bottom position (pedicel side) faces upwards and is more susceptible to higher sunshine exposure. However, the mesocarp fraction (inner peel) has more pore space observed in the bottom than in the mid and top positions on the fruit (**Figure 8.5**) at the end of 42 d of cold storage (7 °C) and after an additional 8 d of shelf storage (23 °C). It is important to note that the exocarp is the outer most fruit tissue and possesses the surface openings used for gaseous exchange between the fruit and the surrounding environment.

In pomegranate fruit, surface coating is one of the applied strategies to control weight loss and chilling injury during postharvest storage (Barman *et al.*, 2011; Meighani *et al.*, 2014; Opara *et al.*, 2015). However, surface coatings tend to create anaerobic conditions by creating an O₂ deficit and high CO₂ atmosphere around the fruit, resulting in the production of fermentative off-flavours (Barman *et al.*, 2011). Therefore, optimisation of surface waxing treatments by differential treatments on the top, middle and bottom of the fruit will help minimise moisture loss without necessarily compromising on the aerobic respiration process of the fruit.

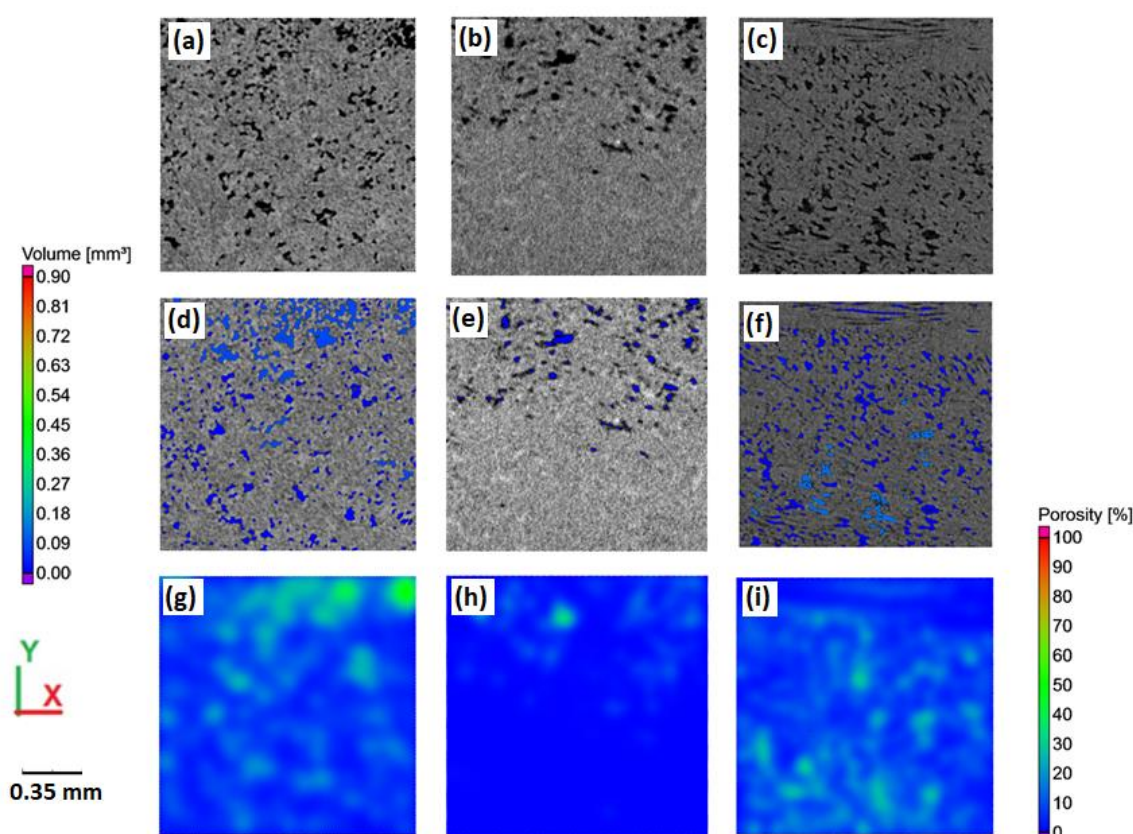


Figure 8.5 Variation of the 2D pore space distribution in the mesocarp (inner peel) fraction with location on the pomegranate (cv. Wonderful) fruit compared at 0 d (before storage). Representative μ CT images from the Top (a), middle (b) and bottom (c) locations on the fruit,

their analysed pore space volume (d), (e) and (f) and porosity distributions (g), (h) and (i), respectively.

8.3.4 Changes in pore space during fruit storage

The intercellular space is reported to increase with fruit development providing pathways for efficient gas exchange between the fruit and the environment (Yamaki and Ino, 1992). The growth of cells facilitates the separation of air spaces from the tissue material resulting in increased volume of the individual pores (Khan and Vincent, 1990). Storage duration and conditions such as temperature and relative humidity which affect fruit weight loss also influence pore space variation within the fruit peel (Kuroki *et al.*, 2004). This is attributed to the resulting mechanical deformation of cells and intercellular space in the fruit tissues due to moisture loss (Aregawi *et al.*, 2014; Fanta *et al.*, 2014). An increase in the percentage of air space with increase fruit mass loss was observed in ‘Fuji’ apples during the storage period of 140 d at 0 °C (Harker *et al.*, 1999). Similar results of increased internal air space with storage duration were observed in ‘Granny Smith’ apples (Tu *et al.*, 1996). In the current study, a consistent increase in pore space is observed in the exocarp peel fraction with increasing storage time, especially in the mid and bottom positions on the fruit (**Figure 8.6**). A change in storage conditions from low temperature (7 °C) to high shelf temperature (23 °C) for an additional period of 8 d, resulted in a tremendous increase in peel pore space, irrespective of the position on the fruit. This is associated with accelerated weight loss resulting from moisture loss due to higher vapour pressure deficit between the fruit surface and the surrounding atmosphere of shelf storage compared to cold storage conditions.

Distinctive results were observed in the mesocarp peel fraction where pore space ratio decreased with storage time and temperature conditions, in the top, mid and bottom positions on the fruit. Quite similarly, a decrease of porosity during a five days shelf storage period at 20 °C and 95 % RH storage duration was reported in cucumber fruit (cv. Mugen) (Kuroki *et al.*, 2004). The mesocarp is made of a softer spongy material and finely compacted cells compared to the exocarp which has a more rigid and firm cell structure. In addition, the mesocarp peel fraction has a higher moisture content of about 76.9 % compared to 64 % in the exocarp fraction of ‘Wonderful’ pomegranate cultivar (Mukama *et al.*, 2018). As a result, the mesocarp region tends to easily collapse in as the fruit loses moisture while the exocarp remains firmer and brittle. The blocking of the gas-filled intercellular spaces of cucumber fruit was attributed to progressive senescence (Kuroki *et al.*, 2014).

8.3.5 Pore space characteristics

Pore space characteristics such as size, shape and orientation have a great impact in aiding or hindering fluid transport within the tissues of the fruit and provide vital information to explain textural properties, gas exchange and water distribution disorders (Dražeta *et al.*, 2004; Herremans *et al.*, 2013). Higher sphericity and compactness factor (closer to one) imply less resistance to fluid flow. The shape characteristics of the pore space were presented in the form of the sphericity (**Figure 8.7**) and compactness (**Figure 8.8**) shape factors. Generally, there were no observable differences in the pore sphericity (**Figure 8.7a**) and compactness (**Figure 8.8a**) within the different peel fractions (exocarp and mesocarp), irrespective of the location on the fruit and storage duration. However, a slight increase in sphericity and compactness was noted in the top and mid locations on the fruit after 42 d of storage at 7 °C. The peel of pomegranate generally has pore sphericity and compactness of 0.57 and 0.29, respectively. The pore sphericity results in this study were higher than the 0.33 and 0.36 observed in unripe and ripened mangoes (cv. Carabao), respectively (Cantre *et al.*, 2014b). Pore sphericity increased with increasing pore diameter (**Figure 8.7b**) following an exponential function. The pore diameter in the peel samples ranged between 0.013 and 0.577 mm before storage which is quite comparable with the 0.05-> 0.5 mm observed in apples and pears (Herremans *et al.*, 2013). Likewise, the smaller the pore diameter, the higher the compactness (**Figure 8.8b**). Similar characteristic pore sphericity and compactness distribution profiles were observed in scanned samples.

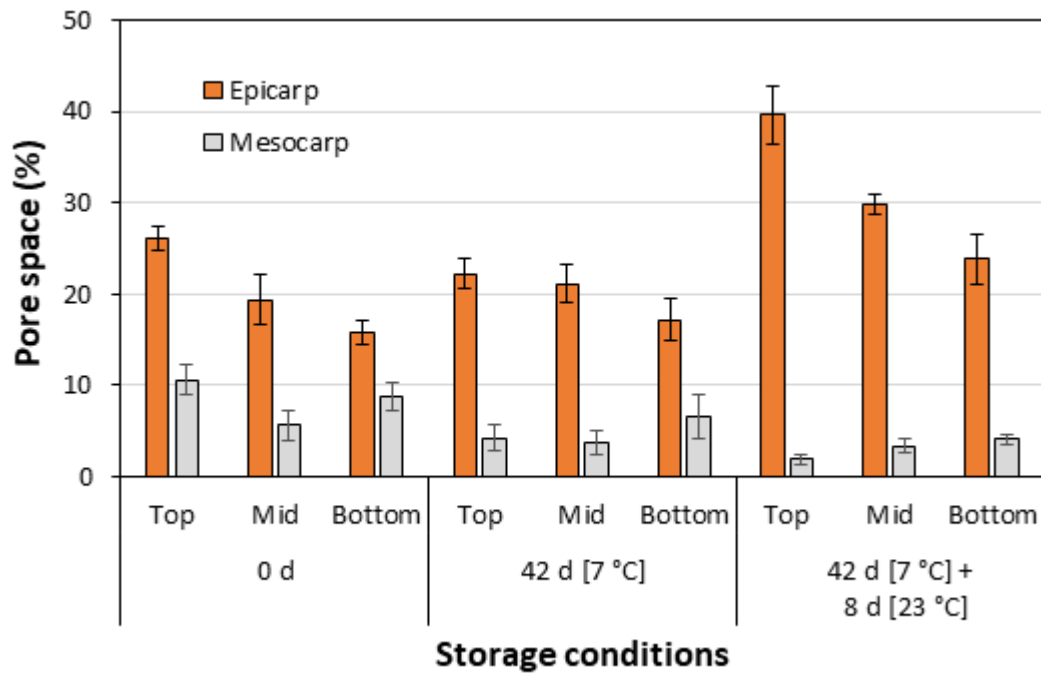


Figure 8.6 Percentage of pore space in the different peel fractions (exocarp and mesocarp) from the different locations (top, mid/middle and bottom) on the pomegranate fruit compared at different storage conditions. The bars represent mean values of six replicates and the vertical lines are error bars at $P \leq 0.05$.

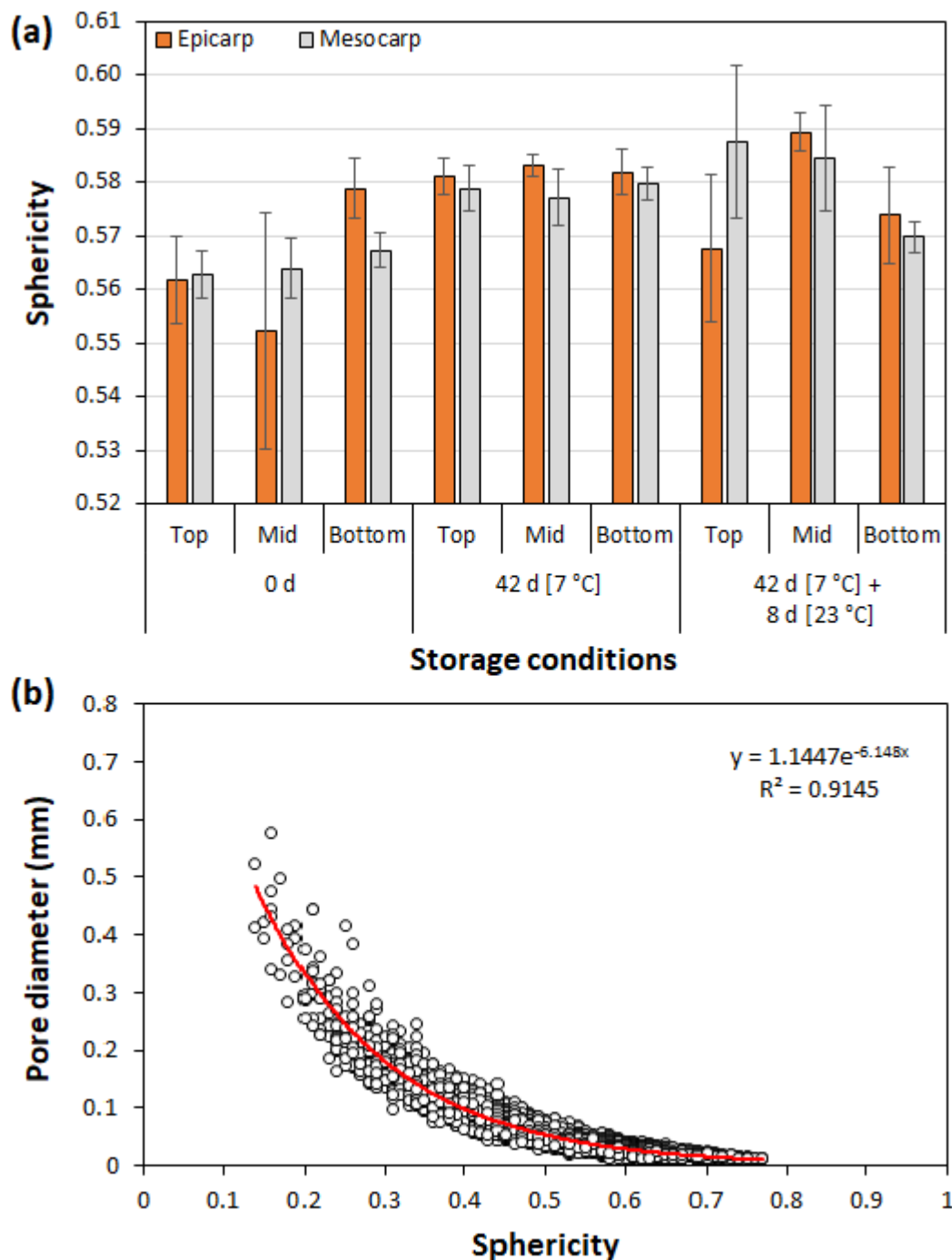


Figure 8.7 Sphericity of pores within the pomegranate peel. The exocarp (outer peel) and mesocarp (inner peel) fractions from the different locations (top, mid/middle and bottom) on the pomegranate fruit compared at different storage conditions (a). The bars represent mean values of six replicates and the vertical lines are error bars at $P \leq 0.05$. A representative sphericity distribution profile with pore diameter (b). The experimental data is represented in the scatter plot while the solid curve fitting is the predicted exponential relationship between sphericity and pore diameter.

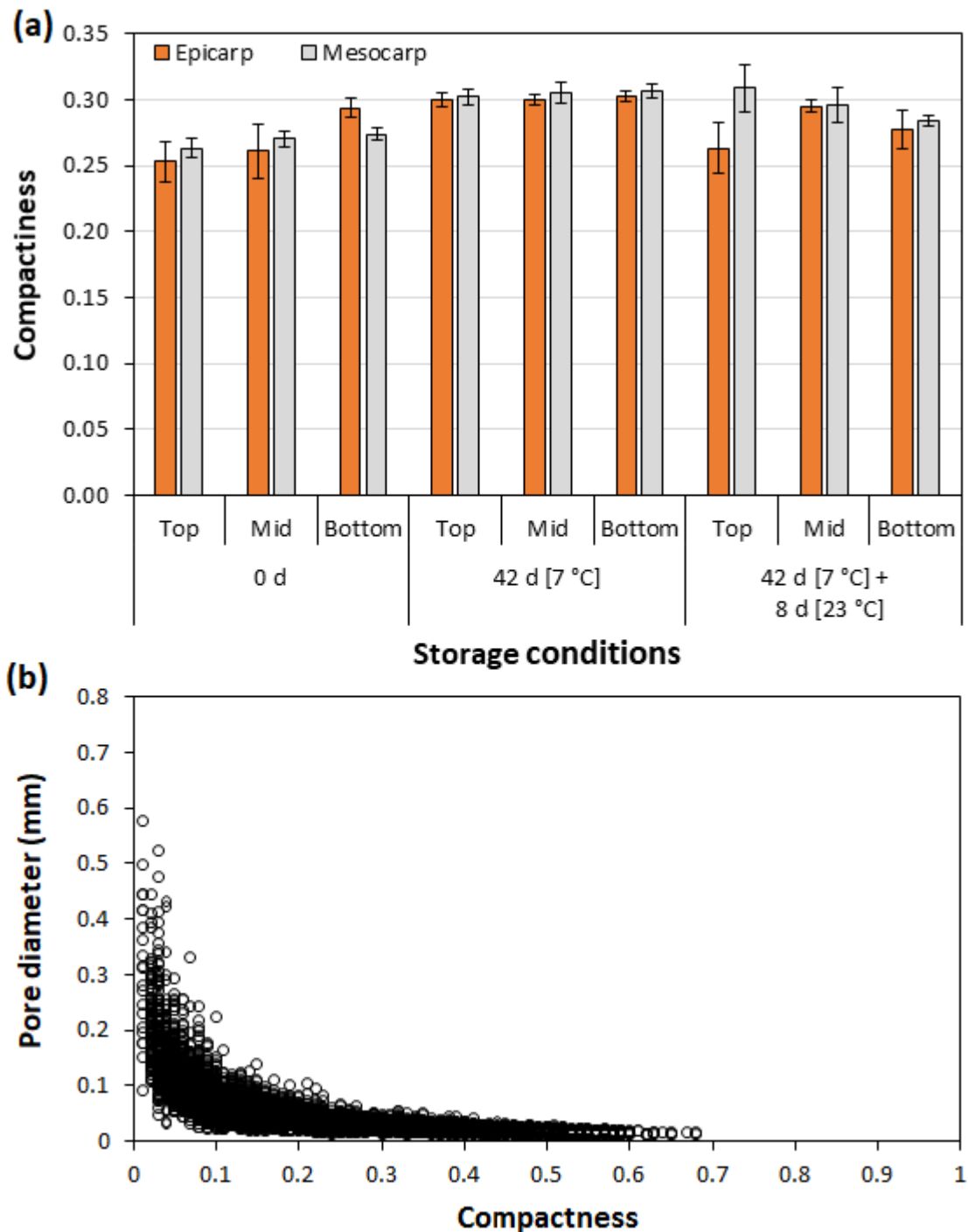


Figure 8.8 Pore compactness within the pomegranate peel. The exocarp (outer peel) and mesocarp (inner peel) fractions from the different locations (top, mid/middle and bottom) on the pomegranate fruit compared at different storage conditions (a). The bars represent mean values of six replicates and the vertical lines are error bars at $P \leq 0.05$. A representative pore compactness distribution profile with pore diameter (b).

8.4 Conclusion

The 3D microstructural pore space ratio, pore distribution, sphericity, compactness and surface area projection within the exocarp and mesocarp peel fractions from the top, middle and bottom locations on pomegranate fruit (cv. Wonderful) were analysed. The changes in these microstructural characteristics with fruit weight loss were examined using X-ray micro-computed tomography.

The study revealed that peel porosity varied on individual fruit depending on tissue type (with higher porosity in the exocarp than in the mesocarp) and location on the fruit (in the exocarp peel fraction, porosity increased from bottom to middle to top locations, while in the mesocarp fraction, higher pore space fraction was observed in the bottom than in the middle and top locations). Peel porosity also increased with storage time as the fruit lost weight in the exocarp fraction, while a decrease was observed in the mesocarp peel fraction.

These findings are relevant in developing strategic postharvest weight loss control techniques in pomegranate fruit. Optimisation of surface coating treatments by differential treatments on the top, middle and bottom of the fruit will help minimise moisture loss without necessarily compromising on the aerobic respiration process of the fruit, consequently preserving its postharvest quality.

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SECTION IV

Modelling water transport in pomegranate fruit and model validation, and the assessment of weight loss control strategies. (Chapters 9-11).

CHAPTER 9

COMPUTATIONAL MODELLING OF THE MOISTURE TRANSPORT IN POMEGRANATE FRUIT UNDER COLD STORAGE AND SHELF LIFE CONDITIONS— PART I: MEASURING THE MOISTURE TRANSPORT PARAMETERS

With regard to Chapter 9, pages 235–253, the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Research, processing of results, and writing of chapter	75

The following co-authors have contributed to Chapter 9, pages 235–253:

Name	e-mail address	Nature of contribution	Extent of contribution (%)
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Declaration with signature in possession of candidate and supervisor	26/02/2020
Signature of candidate	Date

Declaration by co-authors

The undersigned hereby confirm that:

1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 9, pages 235–253,
2. no other authors contributed to Chapter 9, pages 235–253 besides those specified above, and
3. potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 9, pages 235–253 of this dissertation.

Signature	Institutional affiliation	Date
Declaration with signature in possession of candidate and supervisor	Department of Horticultural Sciences, Stellenbosch University	26/02/2020
Declaration with signature in possession of candidate and supervisor	Department of Horticultural Sciences, Stellenbosch University	26/02/2020

Chapter 9

9 Computational modelling of the moisture transport in pomegranate fruit under cold storage and shelf life conditions— part I: measuring the moisture transport parameters

Abstract

The understanding of the heat and mass transfer processes between the surface of produce and the ambient air is vital for the analysis and characterisation of various physiological processes. Methods for quantifying these exchanges have been a subject of active research, helping to explain the function of the different tissue parts, their effects on heat, moisture and gas transport. This aids the understanding of the fruit-environment interaction for a better process and operational control during postharvest storage and refrigerated transportation of fruit and vegetables. Particularly, the peel of pomegranate fruit has an important influence on the moisture and gas transport phenomena. However, the peel structure and its influence on the mechanism of the water loss is still unclear. In this work, X-ray micro-computed tomography was used to visualise the microstructure of pomegranate fruit peel and to quantify the proportion and distribution of the air voids. The Lattice-Boltzmann method (LBM) was employed to model the steady-state permeation of water through the pore space. This procedure generates the water flow velocity field in the pore space. Using the velocity field, the tortuosity and hydraulic permeability of the porous peel sample were calculated. The viscous transport of water through the 3D microstructure was simulated using a direct voxel-based 2-phase material model.

9.1 Introduction

The production and consumption of pomegranate fruit (*Punica granatum* L.) have increased in the past two decades owing to its health and nutritional benefits (Aviram *et al.*, 2008; Guo *et al.*, 2008; Arendse *et al.*, 2014). Following this, research studies on pomegranate fruit have been proliferating (Aviram *et al.*, 2008; Guo *et al.*, 2008). The postharvest handling methods (Opara *et al.*, 2015; Ambaw *et al.*, 2017; Mukama *et al.*, 2017) and health benefits (Aviram *et*

al., 2008; Guo *et al.*, 2008) of pomegranate fruit have been reported. Pomegranate fruit is a highly perishable commodity (Barman *et al.*, 2011a) due to its high susceptibility to water loss (Fawole and Opara, 2013). Water loss causes losses of saleable weight and quality deterioration due to shrivelling. Water loss is controlled by storing the pomegranate fruit at relatively cold temperatures (5-7 °C) and high humidity (relative humidity (RH) > 95%) conditions. Frequently, the industry uses plastic liners to wrap the commodity to control water loss. On the other hand, the high humidity environment causes condensation of free water on the surface of the fruit, accelerating pathogen growth during storage (Lufu *et al.*, 2018). The heat and mass exchange of the commodity with the storage atmosphere is very crucial for a better understanding and control of the humidity and temperature conditions in the storage environment.

Methods to analyse the fruit-environment interactions have been a subject of active research. Analytical, experimental and mathematical models have been used to measure the heat, gas and water transports of various commodity (Veraverbeke *et al.*, 2003; Nguyen *et al.*, 2006a,b; Ho *et al.*, 2011, 2013; Aregawi *et al.*, 2014). Fanta *et al.* (2014) developed a model to describe water transport in a cortex tissue of pear (*Pyrus communis* L. cv. Conference) fruit using a tissue geometry generated by means of a cell growth model. Using this model, the authors calculated the effective water conductivity of pear cortex tissue.

In a different approach, Kuroki *et al.* (2004) used micro casting method and stereological reconstruction of a 3D image from sliced and stained tissue samples have been used to visualise the internal structure of several types of fruit (Kuroki *et al.*, 2004). However, the micro casting technique does not distinguish whether the intercellular spaces are filled with gas or liquid (Kuroki *et al.*, 2004). X-ray computed tomography (CT) is a direct observational method which needs no added artificial material. The intensities and contrasts on an image generated by the CT scan are mainly due to differences in mass density and absorption of the materials that the gas-filled intercellular spaces can be visualised as areas with low intensities. CT has been employed to visualise and characterise airfield intercellular spaces in a variety of plant tissue samples such as mango (Cantre *et al.*, 2014b), kiwi (Cantre *et al.*, 2014a) and cucumber (Kuroki *et al.*, 2004). Herremans *et al.* (2015) visualised the parenchyma tissues of apple (*Malus × domestica* Borkh.) and pear (*Pyrus communis* L.) and successfully characterised the 3D tissue anatomy at the level of single cells and intercellular spaces using this technique. Similarly, Arendse *et al.* (2016) analysed individual pomegranate fruit (cv. Wonderful) and quantified the internal structure of pomegranate fruit. Tanaka *et al.* (2018)

used CT to quantify the internal structure of cucumber fruit during storage based on the average greyscale (GS) value of the CT images. The authors correlated the pick height of the GS to the density and porosity while the standard deviation of the GS value to the moisture content of the fruit.

The anatomical differences in the exocarp of pomegranate fruit have been studied with the use of microscopy (Yazici *et al.*, 2011). This technique gives information of structure of healthy pomegranate fruit peel and proposed to form the basis for further studies related with abnormal peel structure caused by different physiological, pathological and structural problems. The peel of pomegranate fruit plays a vital role in the water and moisture loss of the fruit or the fruit-environment interaction of the fruit in general. However, there are few studies on the heat and mass transport phenomena within the microstructural pore space of plant pomegranate peel. Recently, preliminary studies on water transportation simulation were attempted on the microstructure of pomegranate peel using X-ray μ CT Ambaw *et al.* (2018) and the Lattice-Boltzmann method (LBM). The LBM method is a numerical method used to solve partial differential equations. The LBM method assumes that the fluid is a collection of particles performing consecutive propagation and collision processes over a discrete lattice mesh. Due to this, the LBM has several advantages over the conventional computational fluid dynamic (CFD) methods. The LBM is mesh free method i.e. it can be directly implemented on the voxel data with no need of a meshing software. Therefore, the complex step of mesh creation is avoided which in itself is an error prone procedure due to the required intensive simplification procedures. Especially, for domains with complex boundaries, like the peel microstructure, incorporating microscopic interactions and parallelisation of the solver algorithms of Navier–Stokes based CFD methods are quite difficult (Nourgaliev *et al.*, 2003).

Hence, the first objective of this study was to obtain 3D spatial information about gas-filled intercellular spaces in the peel of pomegranate fruit. For the detection of gas-filled spaces within the tissue, μ CT was applied. Moreover, since the distribution of the intercellular spaces may vary spatially in peel tissues, the second objective of this study was to compare the structural differences of the pore network of peel samples taken from the top, middle and bottom parts of the fruit. The third objective was to apply a direct voxel-based viscous flow simulation to the obtained 3D images as an initial study of permeability of pome fruit peels.

9.2 Materials and methods

9.2.1 Pomegranate fruit sample

Pomegranate fruit (*Punica granatum* L. cv. Wonderful) at commercial maturity were carefully harvested by hand from a commercial orchard situated in Porterville, Wellington (33° 38' S, 19° 00' E), Western Cape Province, South Africa. Fruit were transported in ventilated plastic trays cushioned with paper pads to the postharvest research laboratory, Stellenbosch University. Uniformly sized fruit without defects such as cracks were sorted and packed in dozens inside single layer display type paper cartons, cushioned with paper trays at the bottom.

A total of 24 fruit were used for the analysis. Six fruit were scanned before storage and the remaining 18 fruit stored in a preconditioned cold room at 7 ± 1 °C and 90 ± 5 % RH (relative humidity) for 42 d, mimicking the sea freight duration from South Africa to Europe across the Atlantic Ocean. Six fruit were sampled for scanning at the end of the cold storage period and the remaining 12 fruit were transferred to shelf conditions of 23 ± 3 °C and 58 ± 5 % RH for eight days.

9.2.2 Sample preparation

The cuboid-shaped samples of dimensions $2 \times 2 \times 5$ mm each, were excised from the top (near the calyx), mid (equatorial region) and bottom (near the pedicel) locations on the individual fruit using sharp blades (**Figure 9.1**). Each of the dissected specimens was further sliced into two separate parts: the top part (containing the reddish epidermis part of the peel) and the underneath white fleshy part of the peel (mesocarp). Hence, the scanning was done for the two layers separately. A specimen was immediately wrapped in parafilm to prevent water loss and then fixed to a specimen holder during scanning.

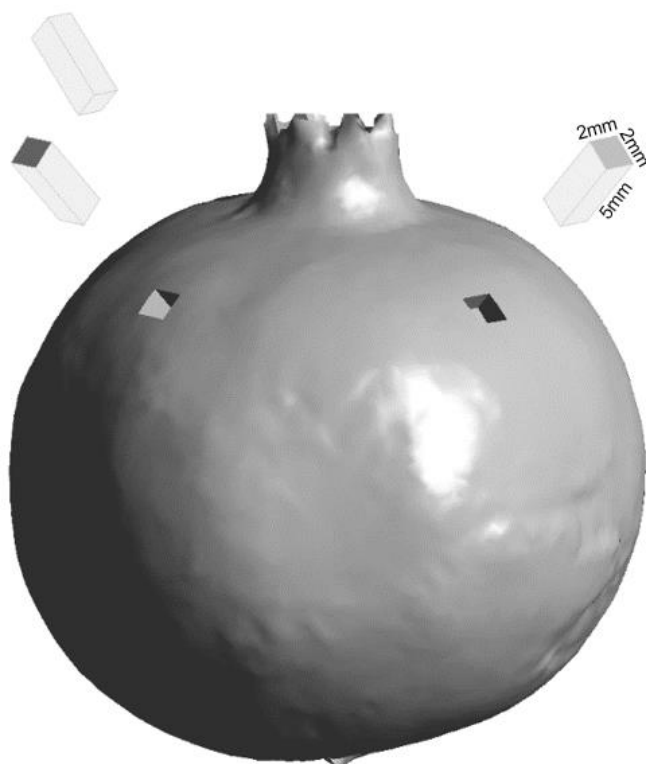


Figure 9.1 Schematic view of the sampling. The samples were approx 2 mm \times 2 mm in cross section 5 mm in height.

9.2.3 X-ray computed tomography (CT) scanner

High-resolution CT was performed at the Central Analytical Facility of the University of Stellenbosch, South Africa (du Plessis *et al.*, 2016b). The system (VjTomejXL240, General Electric Sensing & Inspection Technologies GmbH, Phoenix, Wunstorf, Germany) has an X-ray tube, collimators, turntable and a multi-channel detector mounted into a shielded chamber. A collimated X-ray beam is directed on the sample and the remainder of the ray is intercepted by a multi-channel detector. The detector then transmits a response signal to a computer. The computer analyses the signal and reconstructs a cross-sectional image based on the X-ray transmission data. The scanning was performed using a nano-CT instrument with an isotropic voxel size of 3 μm based on parameter optimisation according to the guidelines in du Plessis *et al.* (2017). X-ray settings included 60 kV and 240 μA , with fast-scan option activated, resulting in total scan times of roughly 1 h per sample, excluding sample setup and subsequent data reconstruction.

The projections obtained from the scan were reconstructed into stacks of 2D 64-bit TIF images using the Phoenix Datos acquisition and reconstruction software (Arendse *et al.*,

2016)(Datos|x@2.1, GE Sensing & Inspection Technologies GmbH, Phoenix|x-ray, Wunstorf, Germany), as seen in **Figure 9.2**. The stacks contained up to 2500 images of cross-sections perpendicular to the exocarp of the sample.

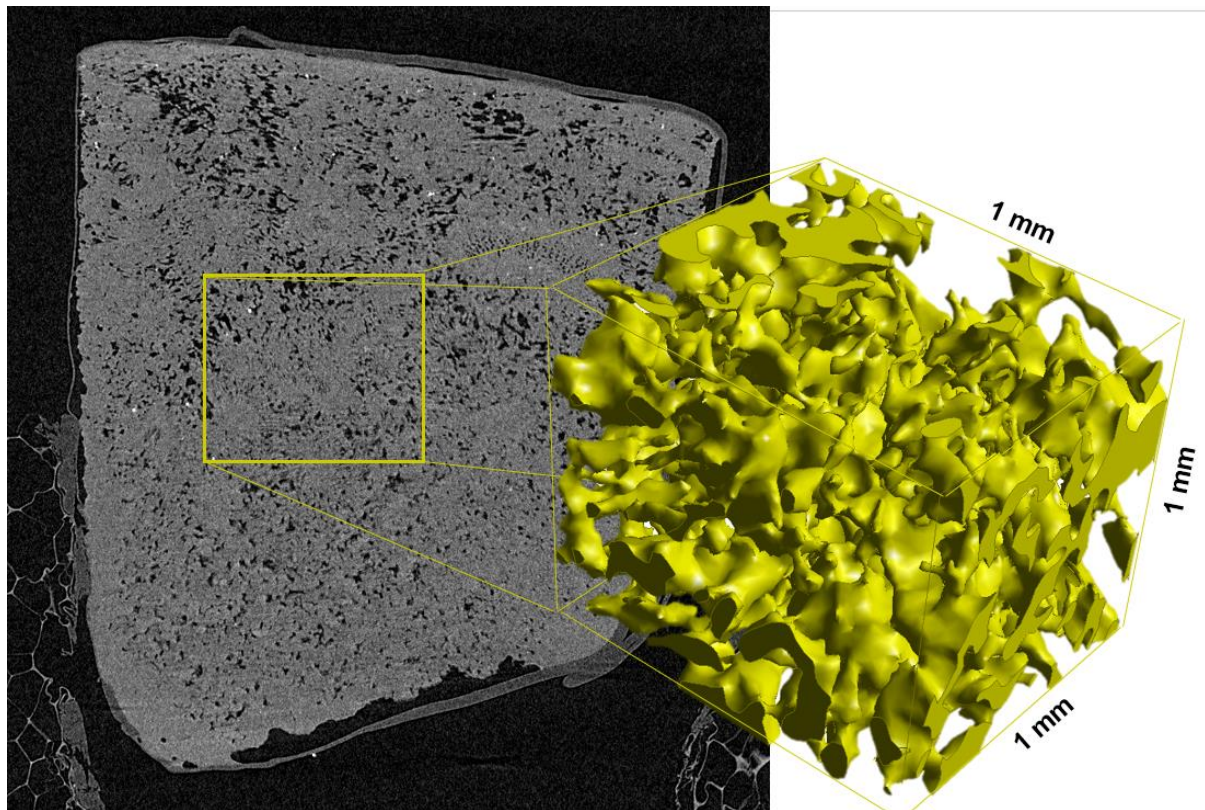


Figure 9.2 Image of the sample peel of pomegranate fruit. The background shows the 2D image in full quality obtained from the X-ray data before any image processing. The cropped image shows the sub-volume taken from the central region of the sample considered for the image and transport process analysis.

9.2.4 Image analysis and statistics

To avoid any surface effects caused by sample preparation and due to the wrapped parafilm, sub-volumes in the centre of the sample was considered (see **Figure 9.2**). The reconstruction was performed with the system supplied Datos software and 3D image visualisation. The primary image analysis and the LBM based water transport simulations were performed in Volume Graphics VGStudioMax 3.2.

Image processing includes an initial de-noising using Adaptive Gauss filter, followed by cropping the volume to remove edge artefacts. This was followed by an advanced surface determination to precisely define the edge between material and void (**Figure 9.3**). This process involves a global threshold, locally optimised at every point to minimise human bias and limit

the effect of brightness changes across the images. This method has been applied with great success previously in porosity analysis (du Plessis *et al.*, 2016a) and for dense inclusion analysis (Le Roux *et al.*, 2015).

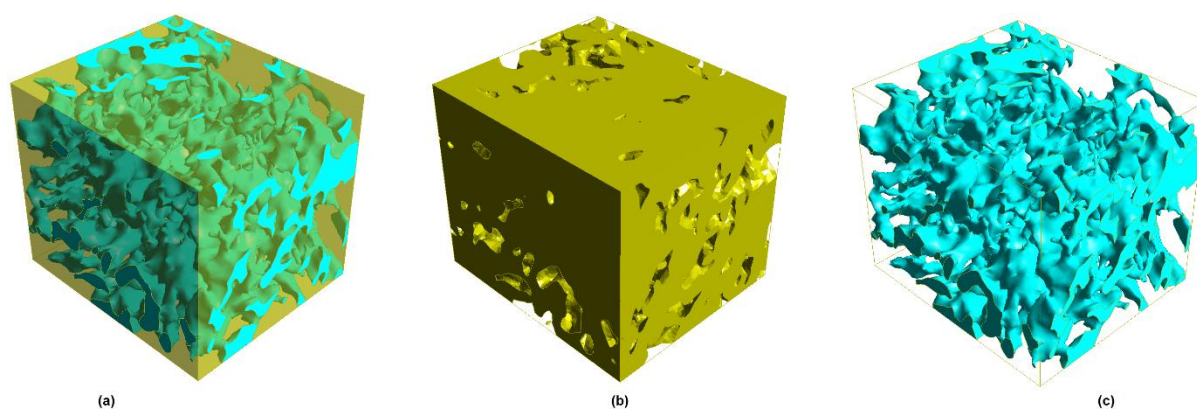


Figure 9.3 Typical 3D geometric model of the peel microstructure ready for the moisture transport analysis (a), the network of the solid matrix (b) and the void space (c).

9.2.5 Modelling the moisture transport using Lattice-Boltzmann method (LBM)

In this study, the Lattice-Boltzmann method with the BGK collision model (Succi, 2001) in the VGStudioMax “transport phenomena” module was used. The LBM is a class of the CFD methods applied in fluid simulations. Unlike other CFD methods, the LBM does not solve the Navier-Stokes equation directly. In this case the LBM method assumes that the fluid is a collection of particles performing consecutive propagation and collision processes over a discrete lattice mesh. With this method, simulation of fluid flow in complex environments such as porous media is quite straightforward because the complex boundaries required in other CFD methods are difficult to work with. The LBM is mesh free method i.e. it can be directly implemented on the voxel data with no need of a meshing software. This method was employed to model the steady-state permeation of water through the pore space by solving **Equation 9.1**. This procedure generates the water flow velocity field in the pore space (**Equation 9.2**). Using the velocity field, the permeability and tortuosity of the porous peel sample were calculated as given by **Equation 9.3** and 9.4, respectively.

The model assumes laminar flow and takes as input fluid viscosity and entrance and exit plane locations, and the pressure difference between the two (**Figure 9.4**). The water transport was modelled for ultra-low-pressure gradient equivalent to 1 Pa to ensure a low flow velocity to mimic a true Stokes flow condition (**Equation 9.1**) inside the pore space.

$$0 = -\nabla p + \mu \nabla^2 V \quad (9.1)$$

where V is the velocity of the fluid, p is the pressure, μ is the dynamic viscosity of water, ∇ is the gradient operator. A pressure gradient of 1 Pa was applied across the two opposite boundaries of the tissue domain (the top and the bottom) while sealing the lateral boundaries (**Figure 9.4**). The absolute permeability k of the material is defined by Darcy's law as given in **Equation 9.2**.

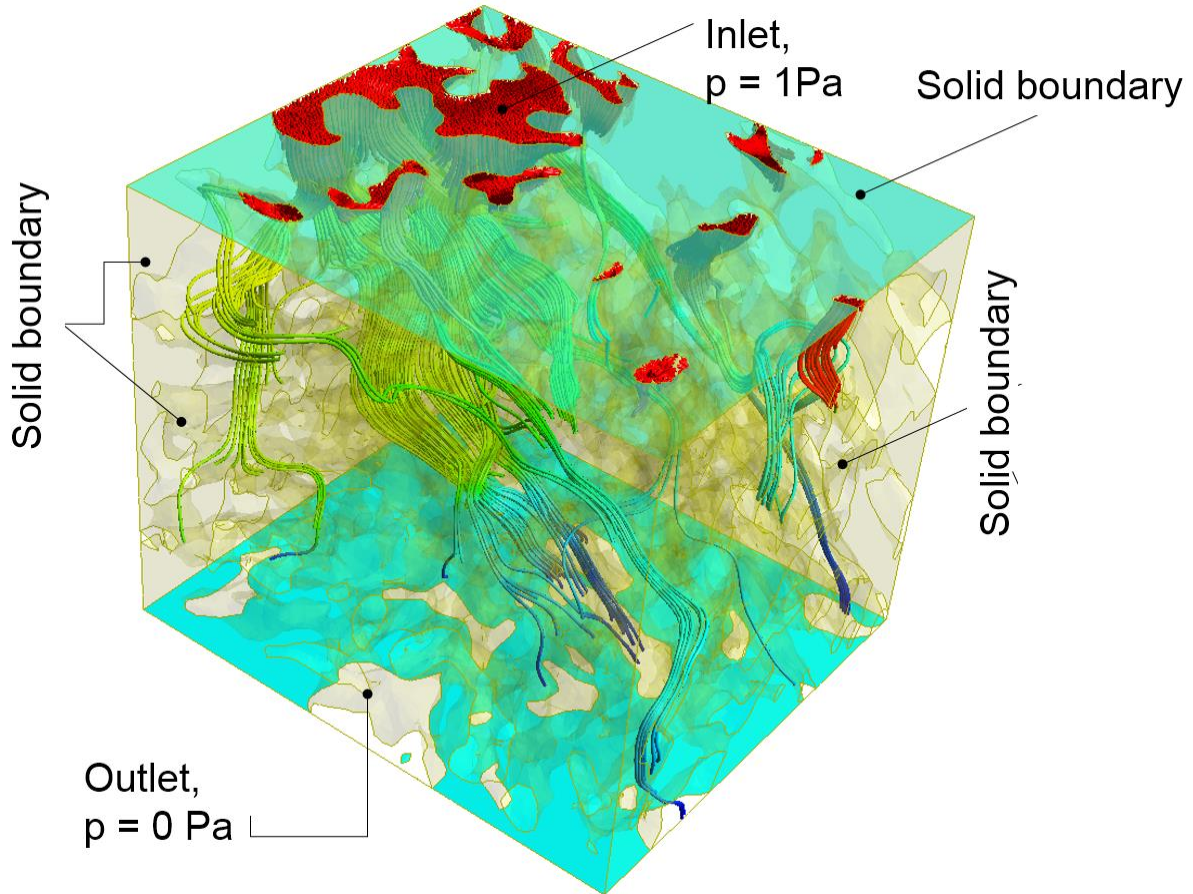


Figure 9.4 The model geometry and the boundary conditions used in the LBM based water transport simulation.

$$\langle V \rangle = \frac{-k}{\mu} \frac{dp}{dx} \quad (9.2)$$

Where dp is the pressure difference between inlet and outlet, dx is the length of the sample (thus, dp/dx is the volume-averaged pressure gradient), μ is the dynamic viscosity of the fluid, and $\langle V \rangle$ is the volume-averaged flow velocity in the medium. In an isotropic medium, the

average flow velocity points in the same direction as the applied pressure gradient. Generally, the average velocity can deviate from this direction, in which case $\langle V \rangle$ is the component of the average flow velocity in the direction of the pressure gradient. According to Darcy's law, the hydraulic permeability, k is computed from the simulation result as:

$$k = \frac{-\langle V \rangle \mu dx}{dp} \quad (9.3)$$

The hydraulic permeability measures the flow resistance exerted by the solid matrix (cells, extracellular matrix) on the flowing fluid and it depends on the detailed geometry and topology of the pore structure.

Porosity was taken as the available void fraction in the 3D microstructure used for the simulation. The tortuosity (τ) is a unitless quantity describing the average elongation of fluid streamlines in a porous medium as compared to free flow. It is the factor by which the length of a typical fluid streamline through the medium deviates from the length of a straight line. It is calculated according to Duda *et al.* (2011) as given in **Equation 9.4**.

$$\tau = \frac{\langle V \rangle}{\langle V_n \rangle} \quad (9.4)$$

where, $\langle V_n \rangle$ is the volume-averaged velocity component in the direction of the pressure gradient.

Once the porosity and tortuosity were obtained, the empirical relation (**Equation 9.5**) was used to quantify the effective diffusivity of moisture in the peel sample.

$$D_{eff} = \frac{\phi}{\tau^2} D_0 \quad (9.5)$$

According to **Equation 9.5**, the effective diffusion coefficient D_{eff} ($\text{m}^2 \text{s}^{-1}$), is proportional to the free diffusion coefficient of water molecule in air D_0 , which takes the value of $2.42 \times 10^{-5} \text{m}^2 \text{s}^{-1}$ at 20°C , but is reduced by a factor which takes into account the reduced cross-sectional area (or porosity, ϕ) available for diffusion and the tortuosity factor τ , of the pore space in the peel structure.

9.3 Results and discussions

9.3.1 Analysis of the pore network

Figure 9.5 and **Figure 9.6** show the pore space distribution on 2D cross-sectional planes through pomegranate peel samples of the exocarp (outer peel) and the mesocarp (inner peel), respectively. The region in the scanned image from where the sub-volume is taken, and the size of the sub-volume is important and was kept consistent between samples to obtain comparable values in the pore network analysis.

The pore space varies with position, especially in the exocarp and is generally highest in the top (near the calyx), followed by the mid (equatorial region) and least in the bottom position (near the pedicel). Average pore space fraction of 29.7 and 5.6 % was observed in the exocarp and mesocarp tissues from the top location, as compared to 22.9 and 4.1 % from the mid location and 18.3 and 6.5 % from the bottom location, respectively. This is probably due to natural mechanisms established during tissue development and fruit growth against excessive transpiration, where regions of the leaves and fruit that are more exposed to sunshine intensity tend to have more compacted cells, fewer surface openings and thicker waxy cuticles (Rehman *et al.*, 2015). The pomegranate fruit are orientated on the tree in a way that the top (calyx side) faces down towards the ground while the bottom position (pedicel side) faces upwards and is more susceptible to higher sunshine exposure. Quite like our results, the intercellular space of 1-5 % was reported in apples and pear (Verboven *et al.*, 2008) however, pore space fraction can go as high as 28-32 % depending on the fruit cultivar (Mebatsion *et al.*, 2006).

The pore space proportion was relatively constant during the cold storage period (**Figure 9.7**). During this period the average porosities of the exocarp samples were 24.2, 20.2 and 16.5 % in the top, middle and bottom sides of the fruit, and 7.4, 4.7 and 7.7 % in the mesocarp samples, respectively. The porosity increased significantly during the shelf life condition, reaching up to 23.9-39.6 % for the exocarp peel samples relative to the location on the fruit. On the other hand, porosity decreased to 1.9-4.1 % in the mesocarp peel samples. The mesocarp is made of a softer spongy material and finely compacted cells compared to the exocarp which has a more rigid and firm cell structure. In addition, the mesocarp peel fraction has a higher moisture content of about 76.9 % compared to 64 % in the exocarp fraction of ‘Wonderful’ pomegranate cultivar (Mukama *et al.*, 2018). As a result, the mesocarp region

tends to easily collapse in as the fruit loses moisture while the exocarp remains firmer and brittle.

In pomegranate fruit, surface coating is one of the applied strategies to control weight loss and chilling injury during postharvest storage (Barman *et al.*, 2011b; Meighani *et al.*, 2014; Opara *et al.*, 2015). However, surface coatings tend to create anaerobic conditions by creating an O₂ deficit and high CO₂ atmosphere around the fruit, resulting in the production of fermentative off-flavours (Barman *et al.*, 2011b). Therefore, optimisation of surface waxing treatments by differential treatments on the top, middle and bottom of the fruit will help minimise moisture loss without necessarily compromising on the aerobic respiration process of the fruit.

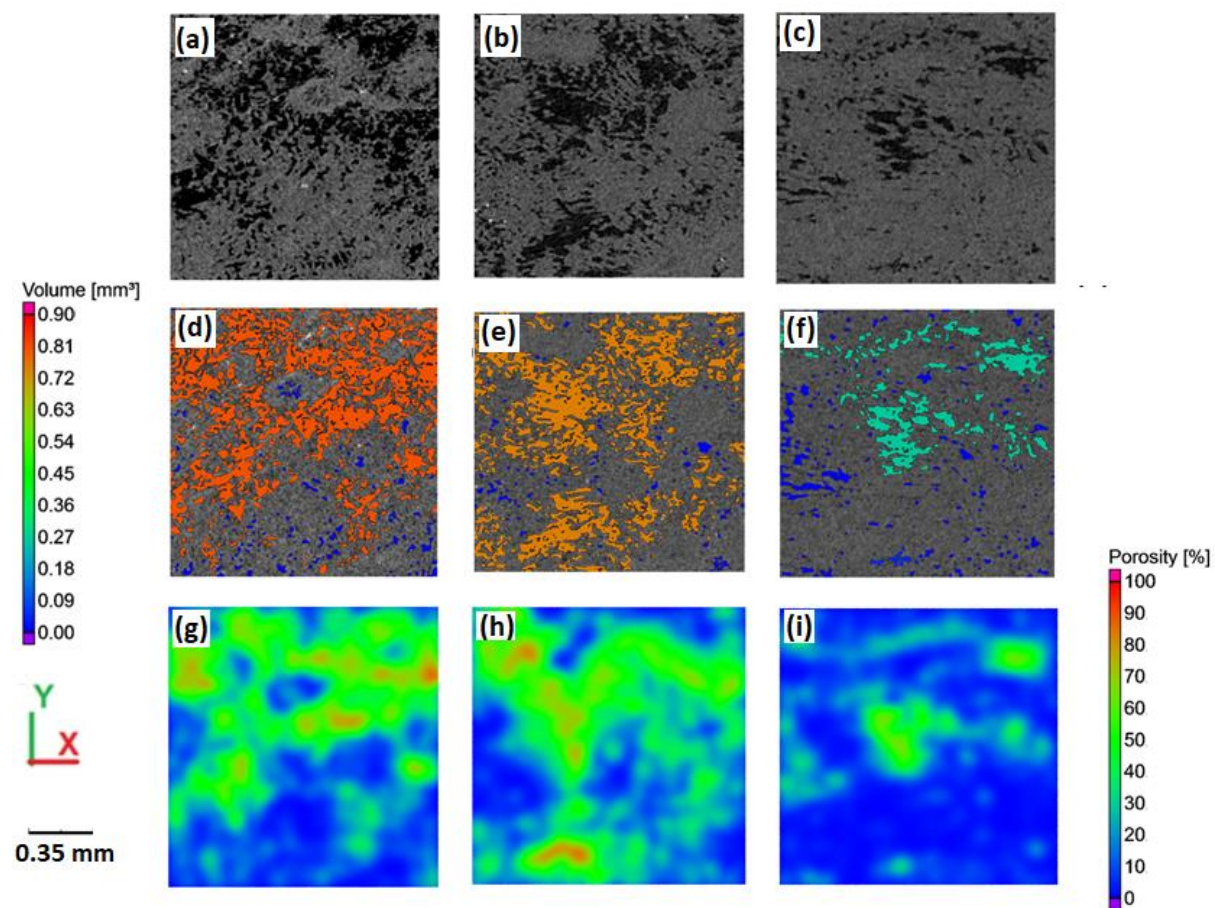


Figure 9.5 Typical CT images of the exocarp (outer peel) sample taken from the top (a), middle (b) and bottom (c) sides of the pomegranate fruit and their analysed pore space volume (d), (e) and (f) and porosity distributions (g), (h) and (i), respectively. Images correspond to pomegranate (cv. Wonderful) fruit at 0 d (before storage).

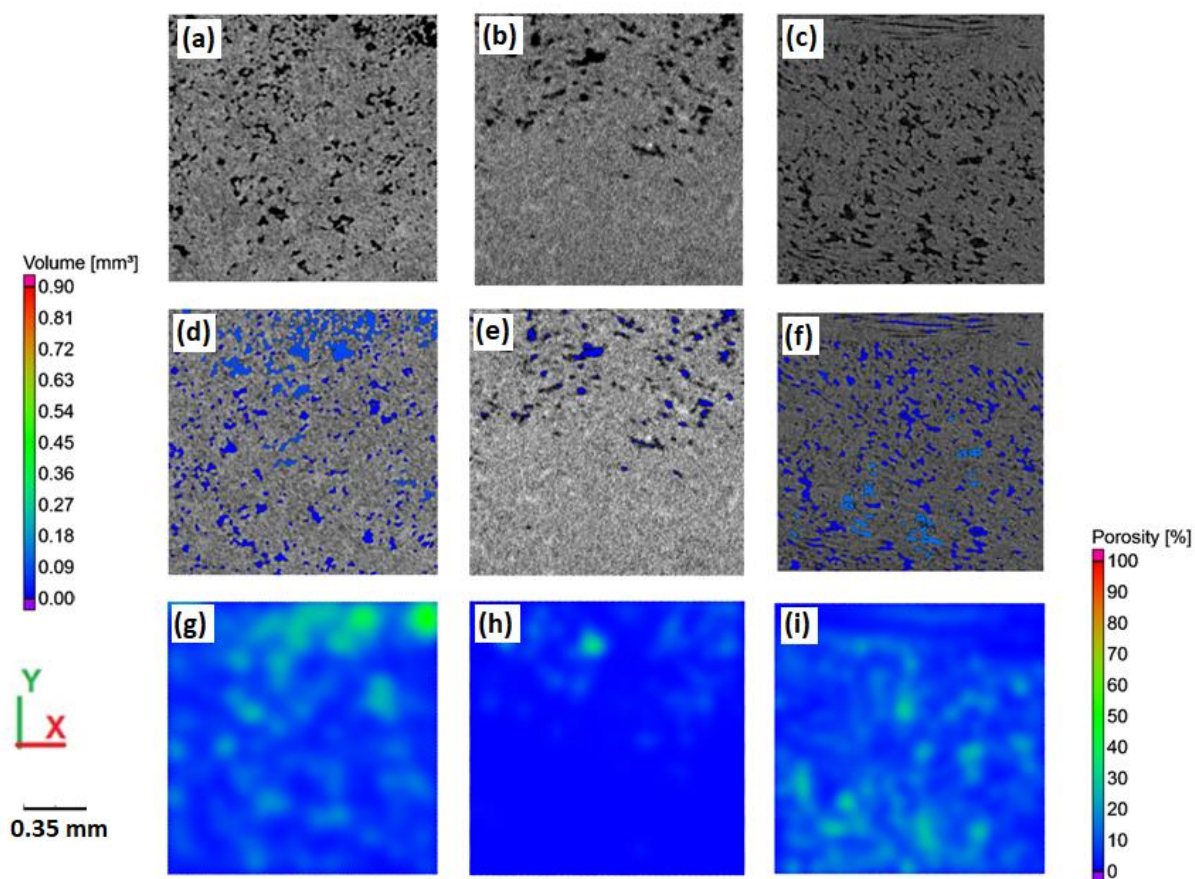


Figure 9.6 Typical CT images of the mesocarp (inner peel) sample taken from the top (a), middle (b) and bottom (c) locations on the fruit and the corresponding pore space volume (d), (e) and (f) and porosity distributions (g), (h) and (i). Images correspond to pomegranate (cv. Wonderful) fruit at 0 d (before storage).

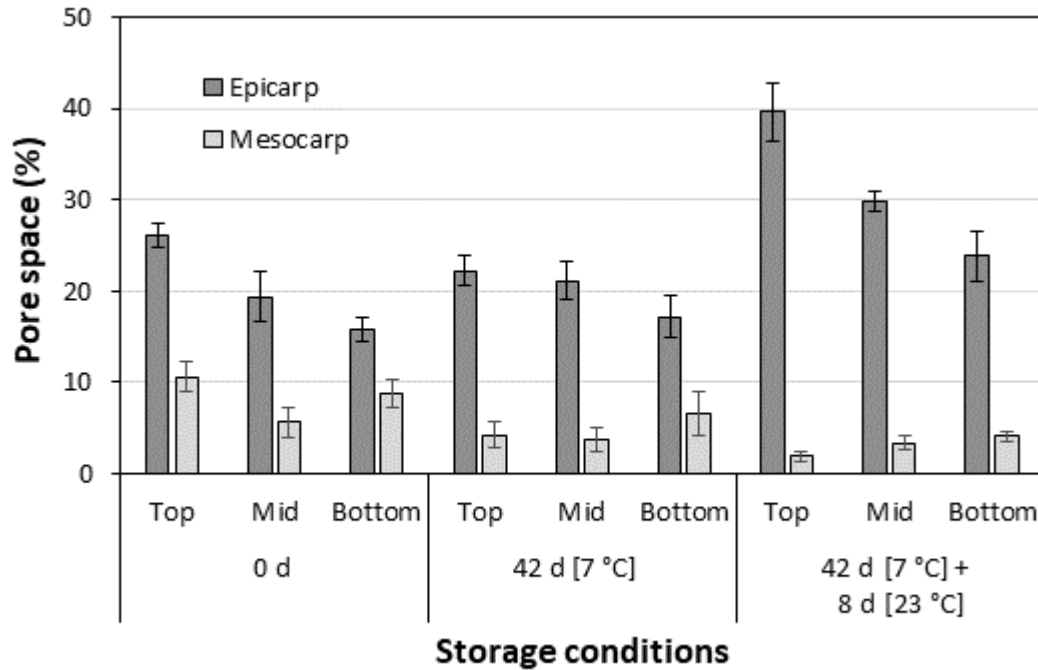


Figure 9.7 Percentage of pore space in the different peel fractions (exocarp and mesocarp) from the different locations (top, mid/middle and bottom) on the pomegranate fruit compared at different storage conditions. The bars represent mean values of six replicates and the vertical lines are error bars at $P \leq 0.05$.

9.3.2 The 3D transient water transport in the peel samples

The absolute permeability measures the ability to transmit fluids (water) through the peel, which is measured in units of millidarcies ($\text{mD} \approx 1 \times 10^{-15} \text{ m}^2$). The absolute permeability of pomegranate peel tissue ranges from 42 mD at the bottom to 500 mD at the top of the fruit for void fraction ranging from 15 % at the bottom to 25 % at the top of the fruit (**Figure 9.8** and **Table 9.1**). The peel sample from the middle of the fruit was always intermediate with a permeability of 212 mD and void fraction of 15 %. The bottom of the fruit is always with a higher value of tortuosity ($= 2.1$) while peels from the middle and top of the fruit were with tortuosity of 1.9 and 1.8, respectively.

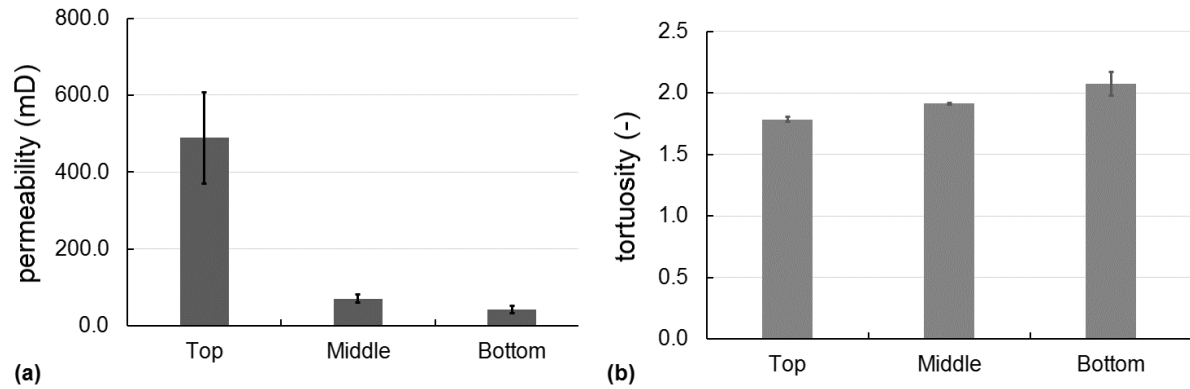


Figure 9.8 Computed value of permeability (a) and tortuosity (b) of pomegranate peel sample. Values indicated are average of simulations performed on three samples taken from three different fruits. The values of computed parameters have negligible differences between fruits (p value $\gg 0.05$) however there are significant differences between samples taken from the three different locations (p-value $\ll 0.05$). Vertical bars indicate standard error.

The pressure and velocity distributions along the flow direction of a typical simulation are shown in **Figure 9.9** (a) and (b), respectively. Notice that there are interconnected pores, while some pores end up without reaching the outlet end of the domain signifying the presence of pore blockages usually measured by specifying the effective void fraction which is in the range of 13.5–38.8 % for the samples analysed, that is 0.8–3.8 % lower than the total pore space measured from the image analysis. Quite similar results are reported by Kuroki *et al.* (2004) on cucumber fruit, where the 3D imaging of gas-filled intercellular spaces revealed that the spaces are not completely interconnected but disrupted in some regions.

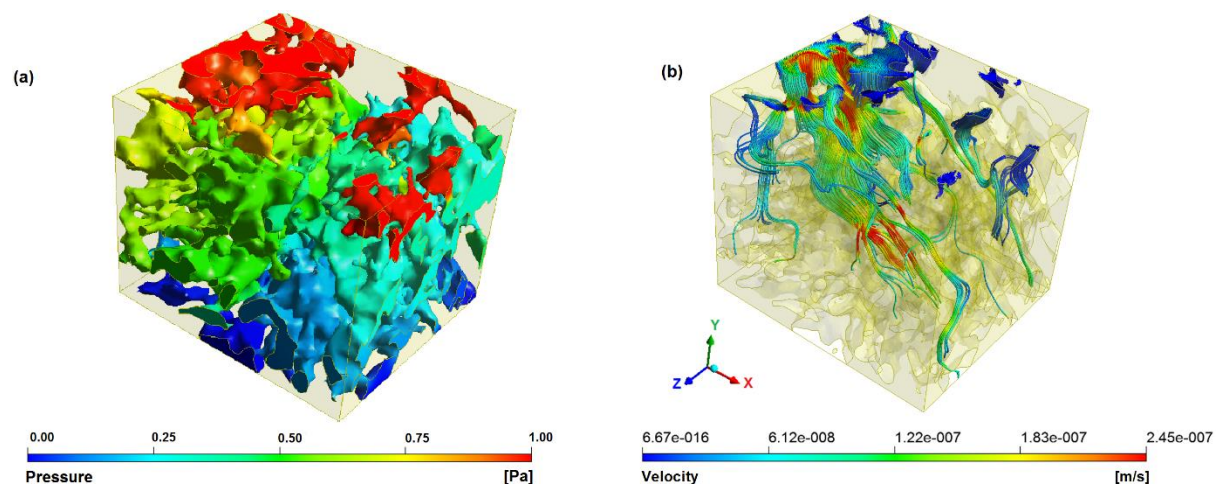


Figure 9.9 Pore-scale pressure distribution (a) and the corresponding velocity field of the viscous flow of water perpendicular to the ZX-plane (b). The pressure and velocity profiles were for across the unit cell ($1 \times 1 \times 1 \text{ mm}^3$).

Table 9.1 Summary of the parameters describing the water transport in the peel of pomegranate fruit

	Storage condition	Porosity (%)	Tortuosity	$D_{\text{eff, model}} (\times 10^{-6} \text{ m}^2 \text{ s}^{-1})$	Hydraulic permeability (mD)
	Initial	20.016	1.785	1.52	354.234
Exocarp	42 d [7 °C]	20.272	1.977	1.26	514.789
	42 d [7 °C] + 8 d [23 °C]	30.253	1.764	2.35	1807.888
	42 d [7 °C]	8.321	2.774	0.26	147.261
Mesocarp	42 d [7 °C] + 8 d [23 °C]	4.860	2.956	0.14	86.010
	42 d [7 °C]	3.083	2.764	0.098	54.562

9.4 Conclusion

The void fraction, permeability and tortuosity were calculated for peel sample taken from the top, middle and bottom region of the pomegranate fruit. The transport of water through the 3D microstructure was successfully modelled using a direct voxel-based 2-phase material model.

Studies showed that waxing of pomegranate fruit surfaces reduces chilling injury and moisture loss during storage (Banks *et al.*, 1997; Opara *et al.*, 2015). However, if waxing is not properly executed, can depress fruit internal oxygen and increases internal carbon dioxide, causing unwanted fermentation (Banks *et al.*, 1997). Hence, it is crucial to control wax formulations and applications. To this end, differential treatment of the top, middle and bottom region of the pomegranate fruit surface, in accordance with their pore characteristics may be considered to optimise the waxing treatment. Further characterisation of the interaction of

initial fruit attributes, coating treatment, moisture loss and other gas transports can be numerically modelled to optimise waxing.

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CHAPTER 10

FINITE VOLUME MODELLING OF THE 3D TRANSIENT WATER TRANSPORT IN POMEGRANATE FRUIT AND MODEL VALIDATION USING MAGNETIC RESONANCE IMAGING (MRI)

With regard to Chapter 10, pages 256–272, the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Research, data collection and processing, and writing of chapter	75

The following co-authors have contributed to Chapter 10, pages 256–272:

Name	e-mail address	Nature of contribution	Extent of contribution (%)
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Declaration with signature in possession of candidate and supervisor	26/02/2020
Signature of candidate	Date

Declaration by co-authors

The undersigned hereby confirm that:

1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 10, pages 256–272,
2. no other authors contributed to Chapter 10, pages 256–272 besides those specified above, and
3. potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 10, pages 256–272 of this dissertation.

Signature	Institutional affiliation	Date
Declaration with signature in possession of candidate and supervisor	Department of Horticultural Sciences, Stellenbosch University	26/02/2020
Declaration with signature in possession of candidate and supervisor	Department of Horticultural Sciences, Stellenbosch University	26/02/2020

Chapter 10

10 Computational modelling of the moisture transport in pomegranate fruit under cold storage and shelf life conditions— part 2: finite volume model development and validation using magnetic resonance imaging

Abstract

The availability of water and therefore its distribution centrally influences the physiochemical quality and behaviour of fresh fruit during postharvest handling. Simulation of water transport within intact pomegranate fruit during shelf storage conditions of 23 °C and 58 % RH were performed for the very first time. A water diffusion model based on Fick's second law was applied through finite volume method (FVM) over the three-dimensional geometry of pomegranate fruit. The model was validated both destructively and non-destructively using gravimetric water content experiment and magnetic resonance imaging mapping, respectively. Generally, a qualitative and quantitative agreement was established between the model predictions and the gravimetric moisture concentration measurements and with the MRI measurements. The accuracy of the model could be improved by accounting for shrinkage of fruit due to excessive moisture loss during shelf storage.

10.1 Introduction

Water is the predominant constituent of fruit existing in different confines of the tissues with respect to the cells. The majority of the water known as intercellular water is found within the confines of the cell membrane, while as cell wall water is locked up in the cell wall, and intercellular water is found within the intercellular space network (Karel and Lund, 2003). Depending on transportability, intercellular, intracellular and cell wall water can be termed as free, loosely bound and strongly bound water (Caurie, 2011). This implies that free water is easily lost to the environment, followed by loosely bound and strongly bound water, respectively. Convection, molecular diffusion and capillary diffusion are considered the predominant mechanisms by which water transport occurs in the food materials, depending on the driving force responsible for the movement (Datta and Zhang, 1999; Nguyen *et al.*, 2006).

Convective mass transfer is driven by a pressure gradient, while molecular diffusion is driven by a concentration gradient. On the other hand, capillary diffusion is influenced by the relative interaction of cohesive and adhesive forces between the liquid and solid phase.

A proper understating of water movement and distribution within living tissues is very influential in explaining the dynamic physical and physiological changes occurring in the fruit during postharvest handling. Water transport and distribution within the fruit tissue and the subsequent mass transfer to the surrounding environment to a great extent influences changes in textural-mechanical properties of the tissues, physio-chemical and sensory attributes of the fruit (Pareek *et al.*, 2015). Information about water transport and distribution in fresh fruit is acquired through experimental and simulation studies. Through modelling, water transport in fresh fruit can be simulated and studied at different spatial scales including cellular (micro-structure scale) level, tissue (mesoscale) level, macro-level (whole fruit scale) and multiscale level (bulk and packed fruit) is promoted to facilitate further understanding of the mechanisms of weight loss (Fanta *et al.*, 2013). This is because fruit are highly heterogeneous and complex in structure. The water profile of plant-based food materials was previously investigated by oven drying techniques (Litchfield and Okos, 1992). The major drawback of this destructive approach is that it is time-consuming and measurements are carried out on different fruit samples throughout the study. As a result, non-destructive techniques have been sought for. A technique based on the absorption of gamma rays was used to investigate a one-dimensional water profile of apples (Chiang and Petersen, 1987). The major drawback for this technique is that gamma rays have an ionizing effect and are not generally safe to work with. On the other hand, magnetic resonance imaging (MRI) is a non-invasive technique that is very safe to work with.

Despite a lower image resolution obtained by MRI system as compared to an electron microscope, high noise frequency during image acquisition and high cost implications, MRI allows for repetitive analysis on the same samples. Though this technique is majorly applied in the medical field, MRI has been embraced as an important non-invasive technique for visualising and monitoring of water transport processes in material science and the food industry. The intensity of the MRI signal is proportional to the number of protons (hydrogen nuclei) in the sample and is often equated to moisture content because the protons are majorly from the water within the sample (Tofts, 2003). Therefore, MRI has been used to acquire temporally and spatially resolved moisture profiles (Bucur, 2003) of materials. For example, 1D, 2D and 3D MRI has been used to detect water core in apples (Wang *et al.*, 1988), estimate

parameters of moisture transport in apple tissues (Verstreken *et al.*, 1998), water transfer in meat (Ruiz-Cabrera *et al.*, 2004) and measurement of bound and free water distribution in wood during water uptake and drying (Gezici-Koç *et al.*, 2017).

In our previous study, we established that weight loss in pomegranate fruit occurs majorly in the peel fraction. In the current study water distribution and transport in the 3D space of pomegranate fruit was for the first time computationally modelled using the finite volume method and validated using MRI water concentration mapping.

10.2 Materials and methods

10.2.1 Fruit acquisition

Pomegranate fruit (*Punica granatum*) of the ‘Wonderful’ cultivar harvested at commercial maturity and ripe stage were obtained from Sonlia Pack House, Wellington, Western Cape (33°38'23"S, 19°00'40"E), South Africa. The fruit had a characteristic deep-red skin and mature deep-red arils (Mphahlele *et al.*, 2016). The fruit obtained were of export-grade having an average medium size of mass size 0.295-0.382 kg and diameter 8.285-9.635 cm. The fruit were transported in an air-conditioned vehicle to the Postharvest Research Laboratory at Stellenbosch University where mass loss monitoring and destructive moisture profile studies were conducted. Non-destructive monitoring of fruit with magnetic resonance imaging (MRI) scanner was conducted at Cape Universities Body Imaging Centre (CUBIC), Groote Schuur Hospital, Cape Town.

10.2.2 Water diffusion model

10.2.2.1 Geometric modelling

Figure 10.1 depicts the geometric model obtained from 3D scanned image of pomegranate fruit on day 0. The space occupied by the arils inside the fruit was approximately created manually using a graphics tool. Hence, the model geometry consists of two separate solid domains: The mesocarp and aril part.

Geometry preparation, domain discretisation and numerical simulations were performed with the CFD code on ANSYS®CFX™ Release 17.0 (ANSYS, Canonsburg, PA, USA). The model geometry was discretised using various mesh sizes (coarse, medium and fine) to perform a mesh independence test. Gradual reduction of the cell size has shown no effect on the results produced beyond about 40,000.

The parameter estimation started by first assuming the effective diffusivity. Setting good initial guess values to speed up the process was accomplished by trial. Several time steps sizes (360 s, 180 s, 72 s, 36 s) were assessed. Based on the accuracy and computational time, time step size of 180 s with a maximum of 10 iterations per time step, was used. Simulation were run on a 64-bit, Intel(R) Xeon(R) CPU E5-1660 v3, 3 GHz, 32 Gb RAM, Windows 7 PC.

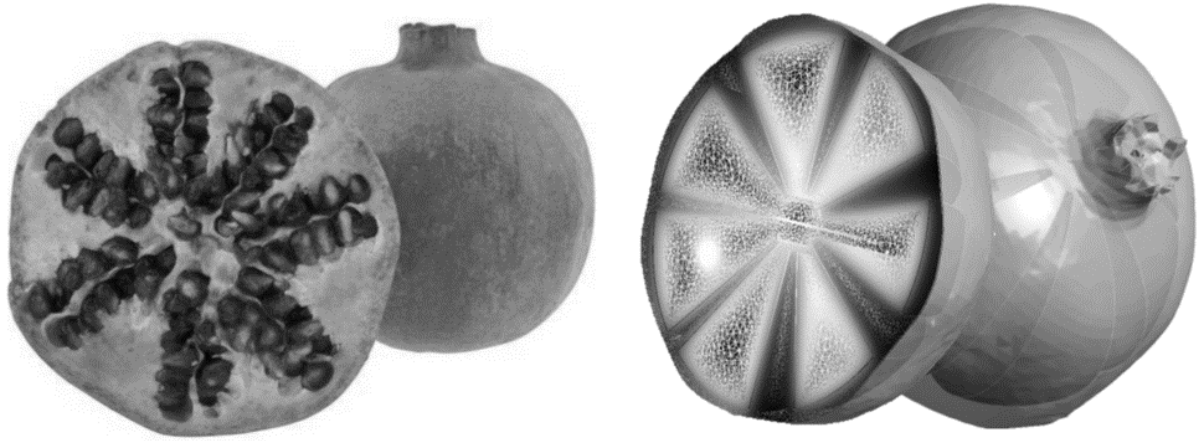


Figure 10.1 Illustration of typical actual pomegranate fruit (left) and the approximating geometry and mesh used in the study (right).

10.2.2.2 Diffusion model

Diffusion of additional variables in solid was used to model the water transport in the model geometry. The fundamental equation to solve is the mass conservation balance for the moisture in the fruit. The water transport is described by a pure diffusion **Equation 10.1**:

$$\frac{dC_a}{dt} = \nabla \cdot D_{eff} \nabla C_a \quad (10.1)$$

where C_a (kg m^{-3}) is the water content per unit volume of the fruit, D_{eff} ($\text{m}^2 \text{s}^{-1}$) is the effective diffusion coefficient, t (s) is the time and ∇ (m^{-1}) the gradient operator.

10.2.2.3 Boundary conditions and model parameters

At the start of the experiment, water content was considered to be independently and uniformly distributed within the different fruit parts/regions with a higher moisture content in the arils than in the mesocarp. At the pomegranate surface, water flux resistance was calculated considering the contribution of outer cortex. The effective diffusion coefficient D_{eff} ($\text{m}^2 \text{s}^{-1}$), is proportional to the free diffusion coefficient of water molecule in air D_0 , which takes the value

of $2.42 \times 10^{-5} \text{ m}^2 \text{ s}^{-1}$ at 20°C , but is reduced by a factor which takes into account the reduced cross-sectional area (or porosity, ϕ) available for diffusion and the tortuosity factor τ , of the pore space in the peel structure. The D_{eff} of the aril domain is initially assumed to be the self-diffusion coefficient of water, which takes the value of $2.3 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$ at 20°C . In an actual situation, the skin of each individual aril will considerably affect the diffusivity and it is not realistic to assume the aril domain as water and assume the self-diffusion coefficient of water. Also, the thin white material covering the entire arils create an added resistance. The diffusivity in the aril domain and the resistance were initially assumed. Comparing the spatio-temporal moisture profile obtained experimentally with that of the simulation model through error minimisation procedure. In this study, the effective diffusivity values estimated for the peel tissues from **Chapter 9** were used in settings up model parameters. Based on the known diffusivity of water in the air, the moisture transfer across the barriers of: the interfaces of fruit-air and aril-mesocarp (albedo) were estimated.

10.2.3 Model validation

10.2.3.1 Dehydration experiment

A separate set of 36 fruit were randomly selected and used in the destructive experiment to monitor moisture profile across the individual fruit. Six fruit were sampled at 0, 3, 7, 10, 13 and 16 d of shelf storage. A cylinder of 2.5 cm diameter was bored through each fruit at the equatorial region. Half of the cylinders were bored right under the aril segments and the other half targeting the region in between the arils to account for variation in peel morphology of the fruit (**Figure 10.2**). The cylindrical samples were then sectioned into thin slices of about 1 mm using a sharp sectioning blade. The thickness of the slices was individually and accurately measured at four opposite positions using a digital Vernier calliper (Mitutoyo, model CD-6 CX, Japan) of accuracy 0.01 mm. The samples of the arils and peel slices were blotted with a paper towel and their moisture content was determined by drying oven method of AOAC 925.45 (AOAC, 2005). The samples were dried in preconditioned Aluminum dishes at $105 \pm 0.5^\circ\text{C}$ for 24 h in a preheated oven (Model 072160, Prolab Instruments, Sep Sci., South Africa) to achieve a constant weight. A profile of water concentration with respect to tissue slice position between the fruit center and surface was then constructed.

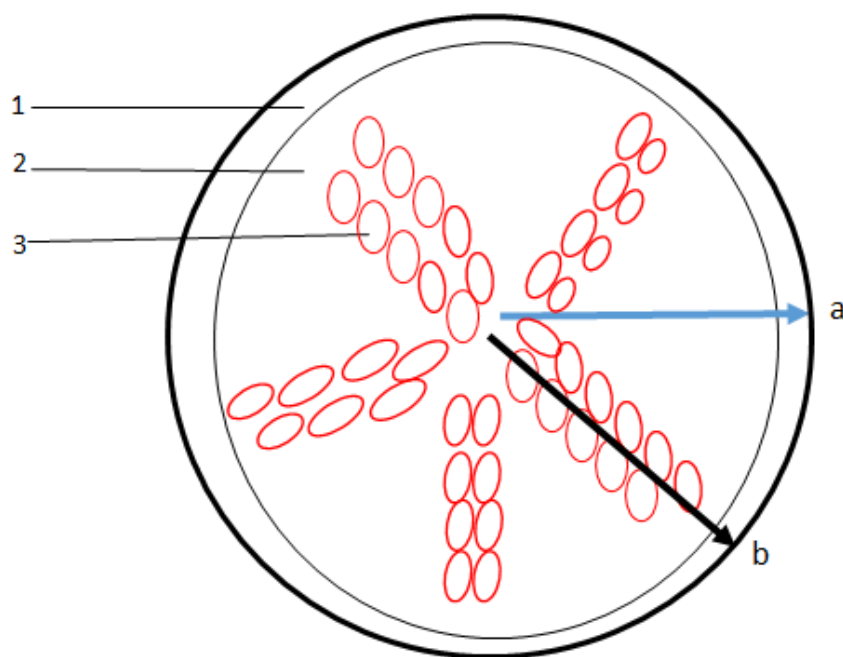


Figure 10.2 Illustration of the studied water profile routes (a) across the peel (non-edible fraction) and (b) across the arils (edible portion) of pomegranate fruit (cv. Wonderful). 1 - exocarp, 2 – mesocarp and 3 – arils (edible fraction).

10.2.3.2 Magnetic resonance imaging (MRI)

Sample preparation

Four fruit of uniform size were randomly selected and used in the non-destructive monitoring of transient water profile across each fruit at 0, 3, 7, 10, 13 and 16 d of shelf storage (23 °C and 58 % RH). Sample holders were moulded by hand from commercial play dough to individually and precisely fit the fruit. The sample holders were made in such a way to accommodate a pair of fruit side by side with a space of 3.5 cm between the fruit (**Figure 10.3**). The fruit and the corresponding moulds were labelled to allow for repetitive placement and positioning.

The 3T whole-body MRI system (Skyra Magnetom, Siemens, Germany) running a VE 11 software version was used in the study. The scanner has up to 204 coil elements and 128 radio frequency (RF) channels to allow for efficient scanning with no coil or subject repositioning required. The sample holders were firmly fitted in the dual (transmit/receive) 15 channel TRx knee coil specialised for high-resolution knee imaging. The set up was then transferred into the whole-body MRI scanner of bore diameter 70 cm. The relative positioning of the samples, sample holders and the knee coil inside the scanner were carefully marked to

allow for easy realignment and repetitive positioning for image acquisition on different experimental days.



Figure 10.3 A pair of pomegranate fruit (cv. Wonderful) placed on a sample holder before scanning with magnetic resonance imaging (MRI) system.

Image acquisition

The images were taken at an irregular time interval applying a multi-echo spin-echo sequence of $TR/TE = 6040/9.5, 19.0, 28.5, 38.0, 47.5, 57.0, 66.5, 76.0, 85.5, 95.0, 104.5, 114.0, 123.0, 133.0, 142.5, 152.0, 161.5, 171.0, 180.5, 190.0$ ms. Scanner and image parameters are summarised in **Table 10.1** below. A multi-echo sequence was chosen to correct for the change of T_2 during drying or due to internal variation within the fruit and to be able to extrapolate the amplitude of the echo signal at $TE = 0$ (Verstreken *et al.*, 1998). The signal corresponding to each of the 20 echoes was sampled and individual separate images were then reconstructed. Therefore, 20 images with different echo times (TE) were obtained per fruit per scan, making it possible to compute the proton density maps and T_2 maps.

Table 10.1 Magnetic resonance imaging (MRI) parameter settings

Scanner and image parameter	Value
Software version	Syngo MR E11
Transmit coil Name	T _x R _x _Knee_15
Scanning sequence	Spin echo (SE)
Sequence Variant	Spoiled (SP)
Scan option	Partial Fourier-Phase (PFP)
MR acquisition type	2D (Frequency × Phase)
Slice thickness	0.8 mm
Total acquisition time	20 min
Repetition time (<i>TR</i>)	6040 ms
Echo time (<i>TE</i>)	9.5, 19.0, 28.5, 38.0, 47.5, 57.0, 66.5, 76.0, 85.5, 95.0, 104.5, 114.0, 123.0, 133.0, 142.5, 152.0, 161.5, 171.0, 180.5, 190.0 ms
Number of averages	1
Imaging frequency	123.26 MHz
Number of echoes	20
Magnetic field strength	3T
Spacing between slices	0.8 mm
Number of phase encoding steps	95
Echo train length	20
Percent sampling	100 %
Percent phase field of view	50
Pixel Band width	650
Acquisition Matrix	[4] 0, 256, 128, 0
In-plane phase encoding direction	ROW (Phase encoded in rows)
Flip angle	180 °
Photometric Interpretation	MONOCHROMES2
Bits allocated	16

Image analysis and data processing

The intensity of the signal in the obtained images greatly depends on the tissue characteristics of the fruit samples as well as the instrumental pulse sequence parameters. Specifically, the signal intensity depends on the dynamic characteristics of the protons in the aqueous phase of the sample often described in the form of the proton density (NH), the spin-lattice relaxation time T_1 (ms) and the spin-spin relaxation time T_2 (ms). Secondly, the signal intensity is also dependent on the pulse sequence parameters of repetition time, TR (ms) and the echo time TE (ms) (Nguyen *et al.*, 2006). In this particular experiment, our pulse sequence involved a high T_R value of 6040ms, therefore the influencing effect of T_1 and T_2 on the signal intensity becomes negligible (Ruiz-Cabrera *et al.*, 2004).

Proton density and T_2 maps were then spatially calculated using T_2 mapping module of the 3D Slicer software (Version 4.10.2 r28257) which is an open-source software application for medical image computing (Fedorov *et al.*, 2012). The module calculates T_2 using a linear least squares method. The mono-exponential decaying signal (**Equation 10.2**) from the T_2 weighted images was natural log-transformed into a straight line (**Equation 10.3**).

$$I_{TE} = I_0 e^{(-TE/T_2)} \quad (10.2)$$

$$\ln I_{TE} = - TE/T_2 + \ln N_H \quad (10.3)$$

Where I_{TE} is signal intensity of a voxel at a specified T_E . I_0 is the signal intensity at echo time at $T_E = 0$ and at this point proton density N_H equals signal intensity I_0 . T_2 is the apparent spin-spin relaxation time constant consisting of both the spin-spin and spin-diffusion effects. Proton density was obtained by extrapolating each pixel of the echo intensity to $TE = 0$. A line plot of the natural logarithm of the signal intensity value of each pixel against the corresponding echo times was constructed (Eq. 1b). T_2 was then obtained as the gradient of the line plot and proton density N_H as the intercept. Proton density of a tissue is the proton concentration in a tissue relative to that in the same volume of water at the same temperature (Tofts, 2003).

Proton density and water profiles

Firstly, the pixel intensity of the images was approximated to be proportional to the water content of the fruit during the experiment (McCarthy *et al.*, 1991; Verstreken *et al.*, 1998). This is because the image intensity is proportional to the mobile water density in the fruit (Wang *et al.*, 1988). Therefore, the proton density obtained from is linearly related to the water concentration in the voxel. The proton density was averaged for every 20 pixels orthogonal to the direction of moisture flow. This was intended to increase the signal-to-noise ratio (Verstreken *et al.*, 1998) and to minimise any possible errors that could arise from fruit repositioning and realignment on consecutive days of data acquisition and to increase the signal-to-noise ratio. The proton density profile from the centre to the surface of the fruit was then established. For comparison, the proton density profiles were transformed into water concentration (kg kg^{-1} on wet basis) profiles by dividing with a specific gravity of the tissues (Whittall *et al.*, 1997). Specific gravity is the ratio of the density of the tissues to that pure water. The density of the exocarp (922.54 kg m^{-3}), mesocarp (950.37 kg m^{-3}) and arils (992.23 kg m^{-3}) of pomegranate fruit (cv. Wonderful) were determined previously (Mukama *et al.*, 2018). Predicted (Finite volume model), experimental (destructive gravimetric validation data)

and MRI (non-destructive validation data) transient water concentration profiles were compared during fruit shelf storage at 23 °C.

10.3 Results and discussion

10.3.1 Simulation of 3D water distribution

The simulated water profiles of pomegranate fruit under shelf storage (23 °C and 58 % RH) are presented in **Figure 10.4**. Generally, a decreasing water concentration with storage duration was predicted in the different regions of the fruit. Before and during the 16 d of storage a higher moisture concentration is maintained in the edible portion (arils) as compared to the non-edible portion (peel). Notice that a steep water gradient rapidly develops within the non-edible portion compared to a slow-developing gradient within the edible portion. Quite similar to these findings, a water distribution model predicted steep gradients underneath the cuticle of pear fruit (cv. Conference) during cold and shelf storage (Nguyen *et al.*, 2006).

In this current study, the model also predicts a higher rate of moisture loss in the region between the arils than the arils region, which is a typical phenomenon of desiccating pomegranate fruit, resulting in inward buckling of the peel surfaces of the former as demonstrated in **Figure 10.5** below. This can be attributed to relatively higher water resistance in the inner epidermal membrane surrounding the arils compared to a higher water permeability in the mesocarp fraction of the fruit peel, as discussed in **Chapter 6**.

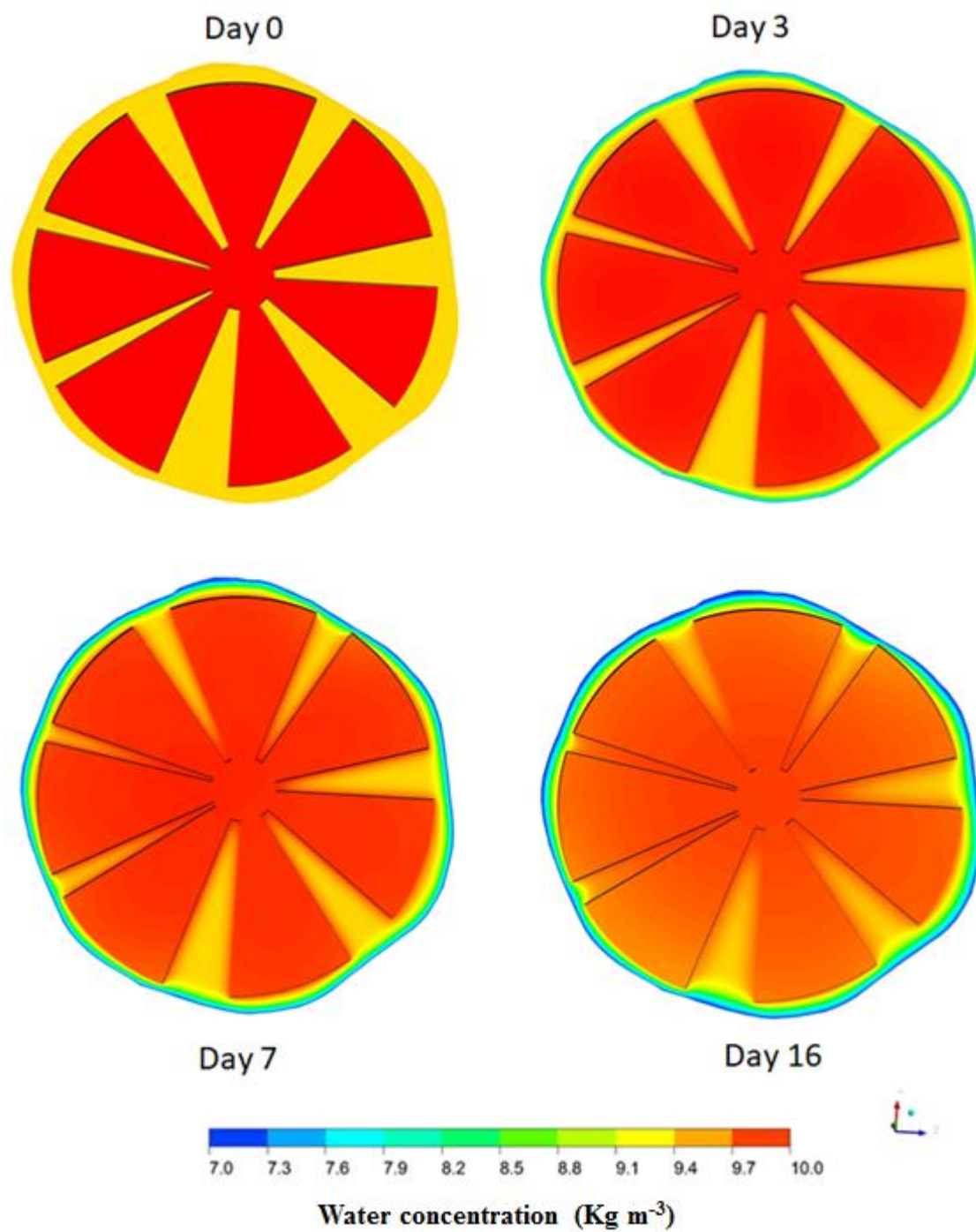


Figure 10.4 Predicted water distribution on a wet basis during shelf storage of pomegranate fruit (cv. Wonderful) at 23 °C/58 % RH for 16 d.

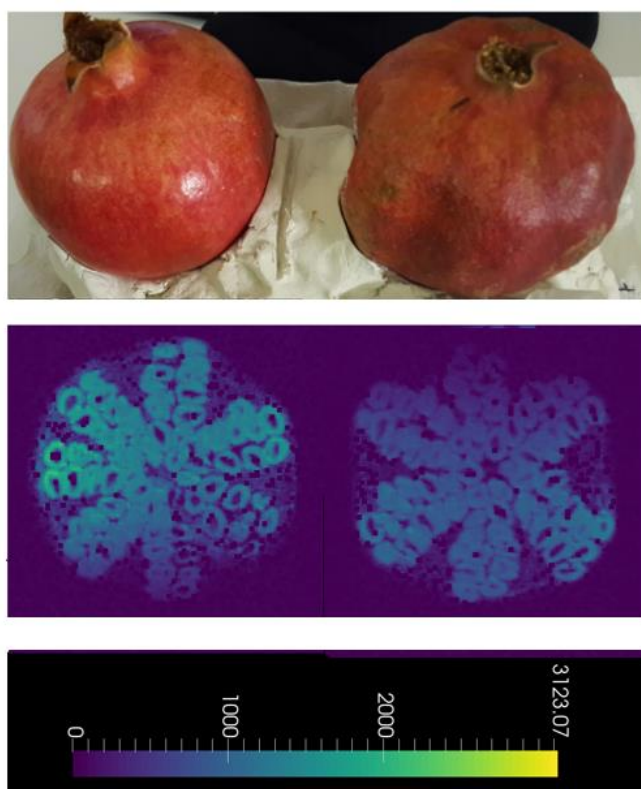


Figure 10.5 Pomegranate fruit (cv. Wonderful) before (left) and after (right) 16 d of shelf storage at 23 °C/58 % RH (Top) and their corresponding proton density maps (bottom).

10.3.2 Validation of 3D water distribution

Figure 10.6 shows water distribution in the form of proton density across the transverse section of the fruit. Proton density is linearly related to the water concentration in the voxel. Therefore, dark regions (low signal intensity) and brighter regions (high signal intensity) represent low and high water content, respectively. The arils appeared brighter than the peel across all treatments, indicating a higher moisture concentration in the edible portion than in the non-edible portion. The seed (source of oil) at the centre of the arils distinctively appeared darker than the rest of the aril region (source of juice). This is because the regions with higher fat/oil appear darker while regions with higher water content appear brighter in T_2 weighted images. Similarly, Nguyen *et al.* (2006) report lower proton density at the seed core than in the cortex tissues of pear fruit (cv. Conference).

Generally, proton density decreased with storage duration especially in the peel as compared to the aril fraction. This is attributed to excessive water loss from the peel fraction as compared to the aril fraction. On the contrary to our finding, Nguyen *et al.* (2006) report unexpectedly increasing proton density in pear fruit during 10 d of shelf storage at 20 °C and

75 % RH, despite a 4.5 % loss in fruit mass. This was attributed to accelerated physical and physiological changes within fruit tissues.

Figure 10.7 the T_2 weighted images of pomegranate fruit at 0, 7 and 16 d of shelf storage. A contrast between the exocarp and mesocarp of the fruit peel was observed before storage in which case the exocarp had a lower signal intensity than the mesocarp. This is attributed to, the lower moisture concentration in the exocarp than in the mesocarp. However, at 7 and 16 d of shelf storage, the exocarp was not easily detectable due to relatively low moisture content as a result of excessive moisture loss.

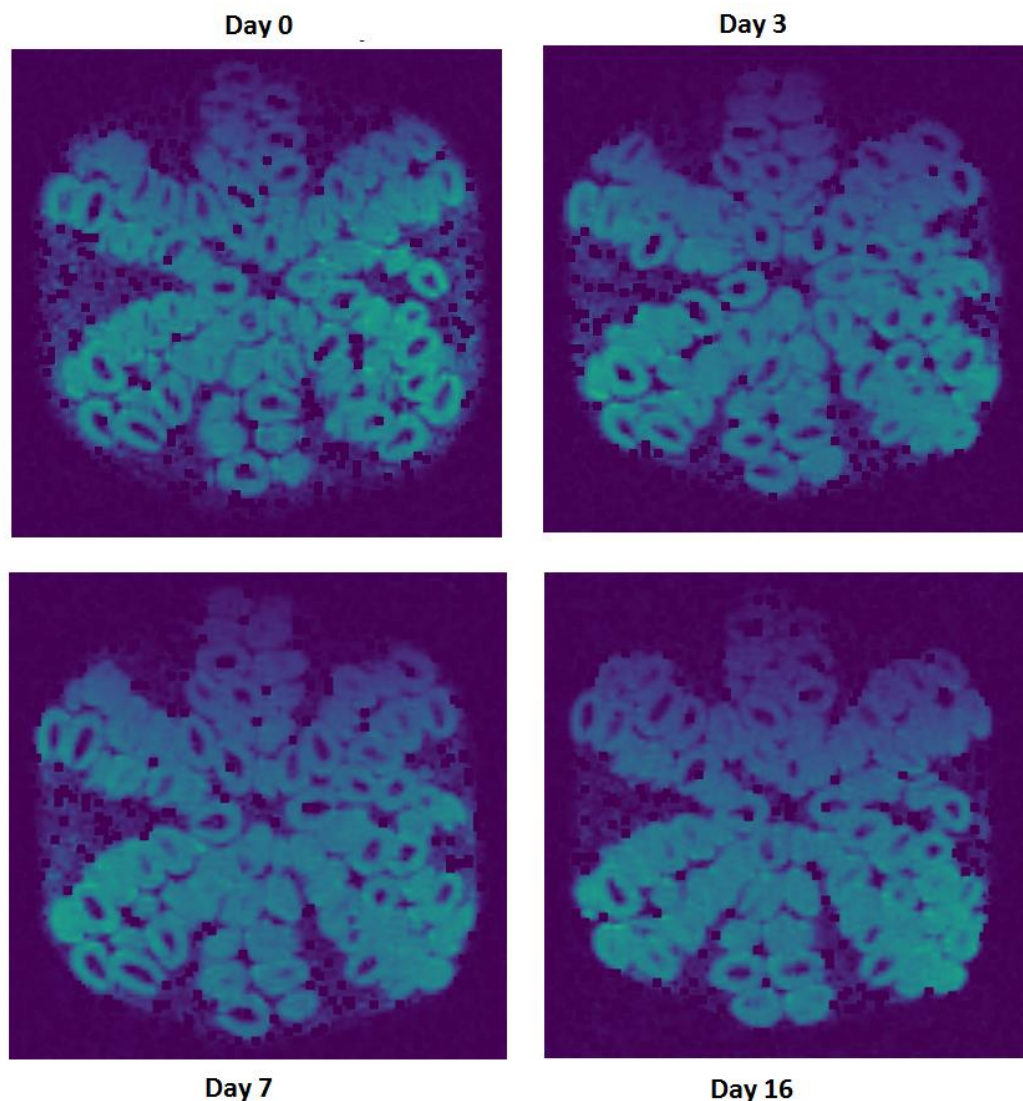


Figure 10.6 Magnetic resonance proton density map of pomegranate fruit (cv. Wonderful) during shelf storage at 23 °C and 58 % RH. The dark regions (low signal intensity) represent lower water concentration and the brighter regions (higher signal intensity) represent regions of higher water concentration. The image contrast represents a decrease in water concentration from day 0 to day 16.

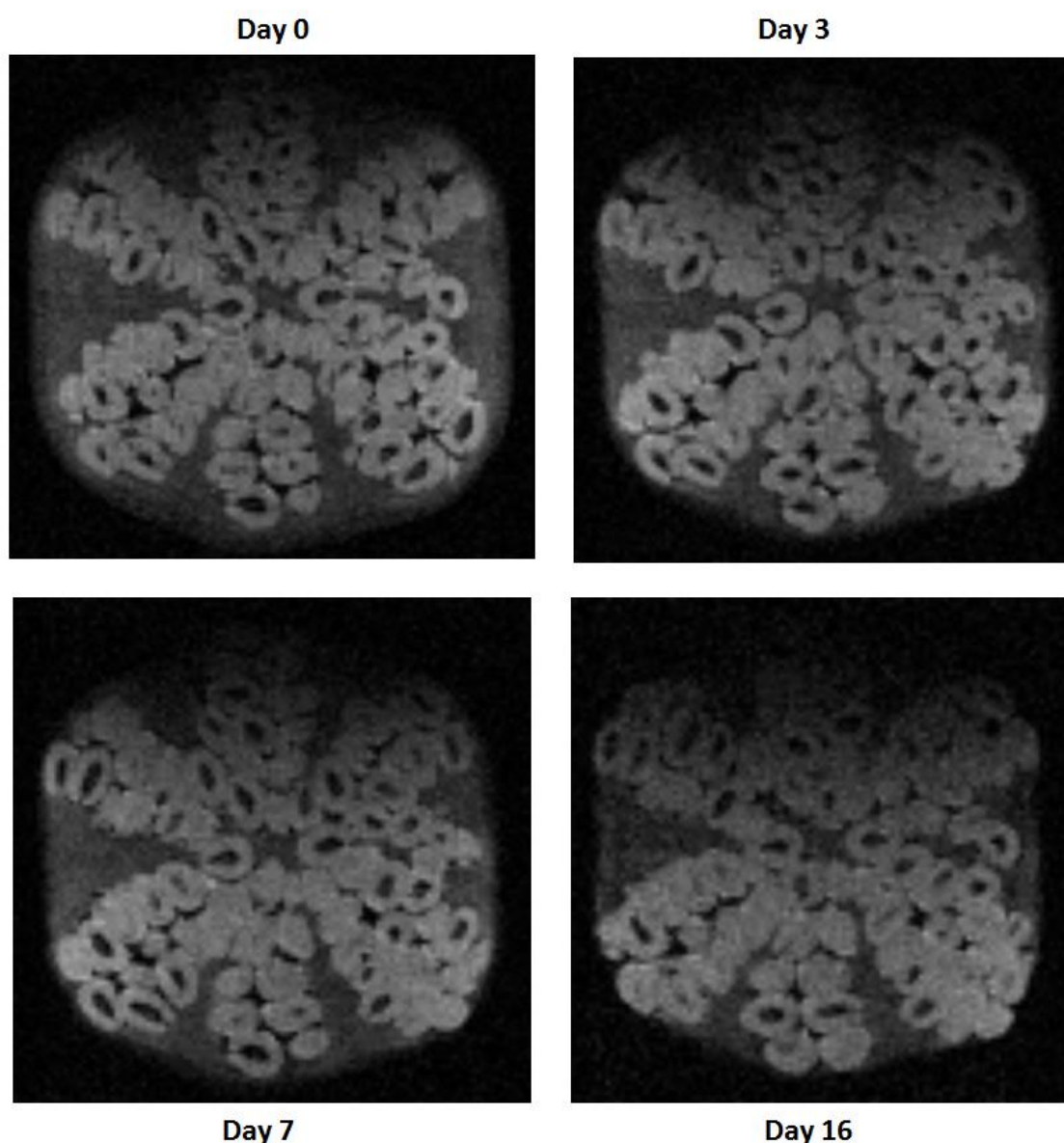


Figure 10.7 Magnetic resonance T_2 weighted maps of pomegranate fruit (cv. Wonderful) during shelf storage at 23 °C/58 % RH. The dark regions (low signal intensity) represent lower water concentration and the brighter regions (higher signal intensity) represent regions of higher water concentration. The image contrast represents a decrease in water concentration from day 0 to day 16.

10.3.3 Transient water profiles

Figure 10.8 shows the predicted (Finite volume model), the experimental and MRI water concentration profiles from the centre to the periphery of intact pomegranate fruit during shelf storage at 23 °C and 58 % RH. For a convenient comparison, the MRI proton density profiles were converted to equivalent water concentration (kg kg^{-1} on a wet basis). A good agreement was established between the predicted, the non-destructive MRI and the destructive gravimetric

experimental results as demonstrated at day 3. The moisture concentration was relatively uniform within the central regions of the fruit however, a steep gradient was noticeable in the periphery regions under the fruit surface. The finite volume model (FVM) predicted a relatively lower moisture concentration at the centre of the fruit than observed through the destructive gravimetric experiment and non-destructive MRI evaluations. However, comparable moisture concentrations between the predicted and the experimental results were noticeable at the surface of the fruit. In this study fruit shrinkage as a result of excessive moisture loss was not accounted for in the model, however, it could help to significantly improve the accuracy of the model.

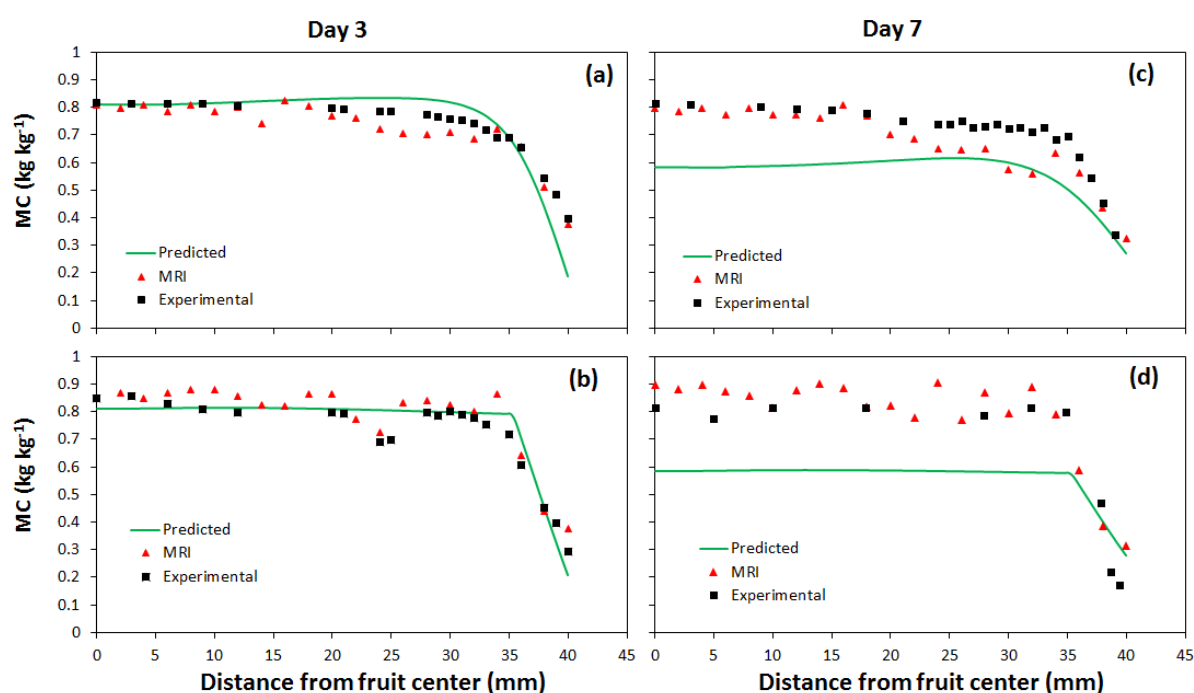


Figure 10.8 The predicted (Finite volume model), the experimental and the MRI water concentration profiles from the centre to the periphery of intact pomegranate fruit (cv. Wonderful) after 3 and 7 d of shelf storage at 23 °C and 58 % RH (a and c) across the non-edible portion and (b and d) across the edible portion (arils), respectively.

10.4 Conclusions

For the first time, the 3D water mapping within the pomegranate fruit was carried out. A finite volume model that incorporates water transport properties of the different pomegranate tissues was used to predict moisture distribution with the fruit during shelf storage conditions at 23 °C and 58 % RH, and transient water concentration profiles were constructed. The model was validated both destructively and non-destructively using gravimetric water content experiment and magnetic resonance imaging mapping, respectively. Generally, a qualitative and

quantitative agreement was established between the model predictions and the gravimetric moisture concentration measurements and with the MRI measurements. Therefore, the developed model is a powerful tool that can be used to study moisture loss of pomegranate fruit during storage, assessing and optimising the application of water loss control strategies. The accuracy of the model could be improved by accounting for shrinkage of fruit due to excessive moisture loss during shelf storage.

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CHAPTER 11

ASSESSING WEIGHT LOSS CONTROL STRATEGIES IN POMEGRANATE (PUNICA GRANATUM L.) FRUIT

With regard to Chapter 11, pages 275–302, the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Research, data collection and analysis, and writing of chapter	85

The following co-authors have contributed to Chapter 11, pages 275–302:

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Declaration with signature in possession of candidate and supervisor Signature of candidate	26/02/2020 Date
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Declaration by co-authors

The undersigned hereby confirm that:

1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 11, pages 275–302,
2. no other authors contributed to Chapter 11, pages 275–302 besides those specified above, and
3. potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 11, pages 275–302 of this dissertation.

Signature	Institutional affiliation	Date
Declaration with signature in possession of candidate and supervisor	Department of Horticultural Sciences, Stellenbosch University	26/02/2020
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Chapter 11

11 Assessing weight loss control strategies in pomegranate (*Punica granatum* L.) fruit

Abstract

Pomegranate fruit is highly prone to moisture loss due to the plentiful micro-pores and slits in the skin, despite having a thick. Water loss results in a huge financial loss to the industry through direct loss of marketable fresh weight and the associated diminished commercial value of affected fruit. Previous studies have investigated the different weight loss control applications almost singly as liner packaging, shrink wrapping or surface waxing with respect to the untreated control. In this present study the different applications of liner packaging, shrink wrapping and surface waxing were evaluated. Secondly, the different methods of waxing application including dipping, brushing and spraying were investigated. Furthermore, fruit were half dipped in wax by dipping only the top or bottom half of the fruit and this was to assess weight loss variation within individual fruit. Batch 1 fruit were stored at 7 °C and 90 % RH for 42 d and thereafter transferred to shelf conditions of 23 °C and 58 % RH for 8 d while Batch 2 fruit were immediately stored under shelf conditions for 16 d. Fruit weight loss, decay incidence, respiration rate, the external total colour difference (TCD), total soluble solids, titratable acidity and sensory quality of arils were investigated. The weight loss of 7.7 % observed in the control fruit at the end of cold storage was minimised by 6.8 and 5.5 % by shrink wrapping, and liner packaging, compared to 2.9, 3.0 and 3.7 % by spraying, brushing and dipping and 1.7 and 2.0 % by bottom-dipping and top-dipping of fruit, respectively. Furthermore, shrink wrapping, liner packaging and wax dipping minimised TCD and preserved the sensory attributes compared to the rest of the treatments.

11.1 Introduction

Pomegranate fruit (*Punica granatum* L.) is highly prone to moisture loss due to the plentiful micro-pores and slits in the skin, despite having a thick rind (Nanda *et al.*, 2001; Opara and Al-Ani, 2010; Fawole and Opara, 2013). A weight loss above 5 % causes shrivelling (Elyatem and Kader, 1984; Lufu, 2017). Even in the absence of any visible shrivelling, water loss can undesirably affect the visual appearance, flavour and textural properties of the fruit (Pareek *et al.*, 2015). Excessive water loss results into browning of the peel and arils and hardening of the

rind (Kader *et al.*, 1984; Artés *et al.*, 2000; Caleb *et al.*, 2012) and huge financial loss to the industry through direct loss of marketable fresh weight and the associated diminished commercial value of affected fruit (Pathare *et al.*, 2013). Weight loss in fresh fruit is influenced by several pre-harvest, harvest and postharvest factors including, orchard practices, canopy position and cultivar effect among others as discussed in the review of the literature (**Chapter 3**). Weight loss may also vary with location on the same fruit owing to variations in the surface waxing coverage, distribution of lenticel, stomata and micro-cracks as proposed in previous studies (**Chapter 6 and 7**).

Different water loss control techniques have been presented and investigated by many researchers. Storage temperature and relative humidity are important control parameters (Arendse *et al.*, 2014; Lufu *et al.*, 2019). Plastic liners and modified atmosphere packaging (Caleb *et al.*, 2013; Selcuk and Erkan, 2014; Banda *et al.*, 2015; Selcuk and Erkan, 2015; Mphahlele *et al.*, 2016; Belay *et al.*, 2018; Lufu *et al.*, 2018), individual shrink wrapping (Nanda *et al.*, 2001; D'Aquino *et al.*, 2010; Mphahlele *et al.*, 2016), waxing and surface coatings (Nanda *et al.*, 2001; Barman *et al.*, 2011; Meighani *et al.*, 2014). These techniques have been applied with great success in minimising the loss of water. However, if not properly used, shrink wrapping and surface coating/waxing can cause anaerobic respiration that leads to producing off flavours (Gil *et al.*, 1996) while plastic liners facilitate moisture condensation within the bags promoting fruit decay (Lufu *et al.*, 2018).

Previous studies have investigated these weight loss control applications almost singly as liner packaging, shrink wrapping or surface waxing with respect to the untreated control (Laribi *et al.*, 1992; Amarante *et al.*, 2001; D'Aquino *et al.*, 2010, 2012; Selcuk and Erkan, 2014, 2015; Lufu *et al.*, 2018; Rao, 2018). However, it is still challenging to perfectly compare results from different studies due to variabilities resulting from geographical differences, cultivar effects and differences in experimental conditions and procedures. Comparative studies with two applications have been considered. For example, Mphahlele *et al.* (2016b) compares both liner and shrink wrap application on pomegranate (cv. Wonderful) under cold storage. In this current study, the aim was to evaluate common industrial postharvest applications in minimising water loss of pomegranate fruit during cold and shelf storage. The different applications of liner packaging shrink wrapping and surface waxing were evaluated. Secondly, the different methods of waxing application including dipping, brushing and

spraying were investigated. Furthermore, fruit were half dipped in wax by dipping only the top or bottom half of the fruit and this was to assess weight loss variation on an individual fruit.

11.2 Materials and methods

11.2.1 Fruit acquisition

Pomegranate fruit (*Punica granatum* L.) of cultivar ‘Wonderful’ were harvested at commercial maturity from a commercial orchard situated in Porterville, Wellington (33° 38' S, 19° 00' E), Western Cape Province, South Africa. Fruit were transported in ventilated plastic trays cushioned with paper pads to the postharvest research laboratory, Stellenbosch University. Sorting of fruit was carried out to ensure size uniformity and that the fruit were free from surface defects such as cracks. Fruit were packed in dozens inside single layer display type paper cartons, cushioned with paper trays at the bottom.

1.1.1 Packaging and waxing

A batch of 576 fruit (48 cartons) were randomly separated into eight lots and each lot of 72 fruit (6 cartons) was assigned the following treatments: (1) the control experiment consisted of fruit with no waxing and no shrink wrapping placed in open-top display type paper cartons without plastic liner bag. (2) passive modified atmosphere packaging (MAP), with fruit enclosed within plastic liner bag (of loading capacity 5 kg, Xtend®, Item code 815-PG28/m, Patent no. 6190710, StePac L.A. Ltd, Israel), and placed inside open top cartons.

(3) shrink film wrapping was carried out using a 19 µm thick co-extruded polyolefin heat shrinkable film (Vector shrink film-19micron Polyolefin (POF) centrefold 1067m, MIPAQ, Durban, South Africa) consisting of five layers of a mixture of low-density polyethylene (LDPE) and polypropylene. Fruit were individually wrapped using a portable I-bar sealer (model: ME-450SP, Mercier corporation, Taiwan) and then the film was heat-shrunked on the fruit using a blowing type and portable heat gun (model: ME-1200-HG, Mercier corporation, Taiwan) with working temperature range 315- 537 °C. Heat was applied for 7 s at a distance of 30 cm while rotating the fruit. The fruit were then placed in open top cartons in dozens.

Surface waxing of fruit was carried using a ready-to-use lac-resin based wax (Endura-Fresh™ 6100, John Bean Technologies Corporation, Cape Town, SouthAfrica) The fruit were first rinsed with tap water and then allowed to dry under ambient conditions (23 °C) for six hours. Different wax application methods were simulated: (4) the whole fruit were dipped in wax for

5 s. (5) only the top half of the fruit was dipped in wax, (6) only the bottom half of the fruit was dipped in wax, (7) fruit were individually brushed thinly with wax using a general-purpose handheld paintbrush and (8) fruit were sprayed with wax using a general-purpose handheld spray can with nozzle aperture 0.55 mm. Care was taken to ensure that fruit were positioned at the same distance from the nozzle and that all sides of the fruit were uniformly sprayed. All waxed fruit was left to air dry under ambient temperatures 23 °C for 12 hours and then placed inside open top cartons in dozens.

11.2.2 Fruit storage

Twelve fruit were used to assess the initial quality of fruit before storage and the 72 fruit of each treatment were stored in two batches each of 36 fruit. Batch 1 fruit were stored at 7 °C and 90 % RH for 42 d and thereafter transferred to shelf conditions of 23 °C and 58 % RH for eight days. This was to mimic the maximum sea freight duration of pomegranate fruit from South Africa to Europe across the Atlantic Ocean, followed by open shelf marketing before consumption. Twelve fruit were selected for quality assessment after 42 d of cold storage and again after an additional eight days of shelf storage. However, fruit packed in plastic (liner and shrink wrap) were removed from their packages when moved to shelf conditions to avoid sudden build-up of moisture condensate within the packages as noticed in preliminary studies (condensate on the roof inside the liners and in the air pockets around the crown inside shrink wraps).

Batch 2 fruit were immediately stored under shelf conditions of 23 °C and 58 % RH. Then twelve fruit were sampled for quality assessment at the eight and sixteen days of shelf storage. This procedure mimics harvested fruit directly placed under shelf storage without cold storage regime.

11.2.3 Measurements and evaluation

11.2.3.1 Fruit weight, external colour change and decay incidence

Twelve fruit were randomly selected from each batch and labelled for weight and external peel colour monitoring. Fruit weight was determined using an electronic scientific scale (Mettler Toledo, model ML3002E, Switzerland, 0.0001 g accuracy). Fruit peel colour was monitored using a digital colourimeter (Minolta, model CR-400, Tokyo, Japan) at the same storage time interval as fruit weight and size. To establish the change in colour, follow up measurements were carried out at the same marked positions on two opposite sides of each fruit. The lightness

(L^*), redness (a^*) and yellowness (b^*) colour properties were measured according to Commission Internationale de l'Eclairage (CIE), 1976. Measurements for fruit weight and peel colour were taken before storage and at intervals of seven days throughout the 42 d under cold storage and afterwards at intervals of two days of under the additional shelf period of eight days of Batch 1. For Batch 2, measurements were taken at two and four days' interval for fruit weight and peel colour, respectively. Fruit decay incidence was visually assessed (Mphahlele *et al.*, 2016; Atukuri *et al.*, 2017). Fruit with visible external decay and or mould growth were counted and discarded at each sampling time.

11.2.3.2 Headspace gas composition

The headspace gas composition (O_2 and CO_2) was determined using a closed system (Caleb *et al.*, 2012). Two fruit were enclosed in an equilibrated hermetically sealed glass jar, in triplicates, for each storage conditions. Measurements were taken before and after two hours using a calibrated handheld gas headspace analyser (CheckPoint, PBI-Dansensor A/S, Denmark) at 23 °C.

11.2.3.3 Chemical attributes

Fresh juice was extracted from the arils using a blender (Mellerware, South Africa). Total soluble solids (TSS) of the fruit juice was measured using a digital refractometer (Atago, Tokyo, Japan). Titratable acidity (TA) of pomegranate juice (PJ) was determined potentiometrically by titration with 0.1N NaOH to an endpoint of pH 8.2 using a compact auto titrosampler (Metrohm 862, Herisau, Switzerland). Titratable acidity was expressed in milligrams of citric acid (CA) per a hundred millilitres of juice.

11.2.3.4 Sensory evaluation

Sensory evaluation was carried out on arils immediately after the fruit were stored for 42 d of cold storage and additional 8 d of shelf storage. A five-member panel of regular consumers familiar with the characteristic taste of pomegranate fruit was selected from the Postharvest Technology Research Group at Stellenbosch (Chen and Opara, 2013; Atukuri *et al.*, 2017). Orientation on specific sensory attributes was given to the panellists (Vázquez-Araújo *et al.*, 2015). Randomly coded samples were presented to the panellists in two batches (four samples each) and the panellists were required to rate the intensity of the sweet taste, sour taste, crispness, off odour attributes and overall score on a 0-4 descriptive scale (0 = none, 1 = slight, 2 = moderate, 3 = much and 4 = very much) (Atukuri *et al.*, 2017).

11.2.3.5 Scanning electron microscopy (SEM)

Samples of the control, dipped, brushed and sprayed fruit were examined under SEM to evaluate the waxing efficiency. This qualitative analysis was carried out in triplicates before fruit storage. A cuboid-shaped sample of dimensions $7 \times 7 \times 3$ mm each, was excised from the mid (equatorial region) location on each of the three fruit using sharp blades. The cuboids were fixed by submerging them in a 2.5 % glutaraldehyde solution buffered with 0.1 M phosphate buffer and stored at 4 °C overnight. Samples were washed three times for ten minutes in 0.1 M phosphate buffer (at a pH of 7.2). Post-fixation was carried out with 1 % osmium tetroxide in 0.1 M phosphate buffer at room temperature in light-proof vials for two hours. Osmium tetroxide has good fixative and excellent stain abilities for lipids in membranous structures. Samples were then washed three times for ten minutes with distilled water. The samples were slowly dried between filter paper in an oven at 37 °C for 48 hours, instead of the conventional dehydration using ethanol and hexamethyldisilazane (HMDS) to minimise the destabilisation and loss of the natural surface wax. The dehydrated samples were mounted on metal specimen stubs, sputter-coated with gold for 3.5 min using a sputter coater to improve electron emission of the samples.

11.2.4 Calculations

11.2.4.1 Weight loss

Cumulative water loss was calculated with respect to the unit fruit mass (**Equation 11.1**).

$$WL = \frac{(m_i - m_t)}{m_i} \times 100 \quad (11.1)$$

Where; WL is water loss per unit fruit mass (%), m_i (g) is the initial fruit mass, m_t (g) is the mass of fruit after storage days.

11.2.4.2 Respiration rate

The respiration rate (RR) was calculated in terms of oxygen consumption rate (R_{O_2}) and carbon dioxide production rate (R_{CO_2}) in $\text{mL kg}^{-1} \text{h}^{-1}$ by fitting experimentally obtained data into **Equation 11.2** and **11.3**, respectively (Caleb *et al.*, 2012). Respiration quotient (RQ) was then calculated as the ratio of carbon dioxide production rate to oxygen consumption rate of the fruit (**Equation 11.4**).

$$R_{O_2} = 10 \times \frac{V_f}{m} \times \left(\frac{C_{O_{2i}} - C_{O_{2t}}}{t - t_i} \right) \quad (11.2)$$

$$R_{CO_2} = 10 \times \frac{V_f}{m} \times \left(\frac{C_{CO_{2t}} - C_{CO_{2i}}}{t - t_i} \right) \quad (11.3)$$

$$RQ = \frac{R_{CO_2}}{R_{O_2}} \quad (11.4)$$

Where $C_{O_{2t}}$ and $C_{O_{2i}}$ are concentrations (%) of O_2 at a time t (h) and initial time t_i (h), respectively. Likewise, $C_{CO_{2t}}$ and $C_{CO_{2i}}$ are concentrations (%) of CO_2 at a time t (h) and initial time t_i (h), respectively. In this study $(t - t_i)$ is constant and equals to 2 h. V_f is the free volume (mL) in the jar which is the total volume minus the volume occupied by the fruit and m (g) is the mass of fruit inside the jar, the constant 10 is a unit conversion factor ($g\ kg^{-1}$).

11.2.4.3 Total colour difference

The change in external peel colour was calculated as total colour difference (TCD) using **Equation 11.5**.

$$TCD = ((L^* - L^*)^2 + (a^* - a^*)^2 + (b^* - b^*)^2)^{1/2} \quad (11.5)$$

Where L^* , a^* and b^* are the reference values and L^* , a^* and b^* are the respective values of lightness, redness and yellowness colour parameters at a given time (Pathare *et al.*, 2013). Fruit decay incidence was calculated using **Equation 11.6**.

$$Decay\ incidence = 100 \times \frac{n_d}{N} \quad (11.6)$$

Where N is the total number of fruit in carton ($N = 12$) and n_d is the number of fruit with visible external decay and or mould growth.

11.2.5 Statistical analysis

Measured and calculated data on fruit physical and physio-chemical attributes were analysed using Statistica software (Statistica 14.0, Statsoft, USA). The data was also subjected to analysis of variance (ANOVA) to assess the main effects of weight loss control treatments and storage duration. A Duncan's Multiple Range Test was carried to test for statistical significance at $p < 0.05$.

11.3 Results and discussion

11.3.1 Weight loss

Figure 11.1a-c shows the weight loss profiles of pomegranate fruit under different weight loss control treatments (packaging and waxing) and storage conditions. The main experimental factors of treatment, storage conditions (cold and shelf storage) and storage duration as well as their interaction significantly ($P < 0.05$) influenced fruit weight loss. Generally, other treatments greatly minimised fruit weight loss relative to the no-wax-no-package control across all tested conditions. Weight loss was highest in control fruit (7.7 %), followed by bottom-dip waxed (6.0 %), top-dip waxed (5.8 %), wax sprayed (4.8 %), wax brushed (4.6 %), wax dipped (4.0 %), liner packaged (2.1 %) and least in shrink wrapped (0.8 %) fruit, by the end of the 42 d of cold storage. A similar trend is observed during the subsequent additional eight days of shelf storage as well as during the independent 16 d of prolonged shelf storage. A weight loss of 19.8, 15.2, 14.8, 14.0, 14.0, 11.2, and 0.9 % was observed in the control, bottom-dipped, top-dipped, wax sprayed, wax brushed, wax dipped and shrink wrapped fruit, respectively at the end of the 16 d of prolonged shelf storage.

A significantly higher weight loss was observed in all waxed fruit as compared to the packaged fruit. This is attributed to higher water vapour permeability and diffusivity in the wax material than in the plastic liners and shrink wrap. In waxed fruit, weight loss was lower in dipped fruit than in half waxed (top and bottom dipped), brushed and sprayed fruit. This is attributed to differences in wax thickness resulting from the different application methods. Spraying and brushing methods result in thinner wax deposits and coverage compared to fruit dipping. This is discussed further in **Section 11.3.8** below. The slightly higher weight loss in the bottom waxed as compared to the top waxed fruit is attributed to more surface micro openings (lenticels) on the top side of the fruit than on the bottom side of the fruit as observed and discussed in **Chapter 7**. This is further buttressed by findings from **Chapters 8** where a higher porosity (void space) was noticed in the top than in the bottom location samples of the fruit peel.

Individual shrink wrapping was the best treatment in minimising weight loss in pomegranate fruit compared to plastic liner packaging using (MAP) and surface waxing under cold and storage conditions. This is attributed to the combined effect of the high resistance (barrier) properties against moisture diffusion in the shrink wrap plastic film and its ability to block the surface opening of the fruit.

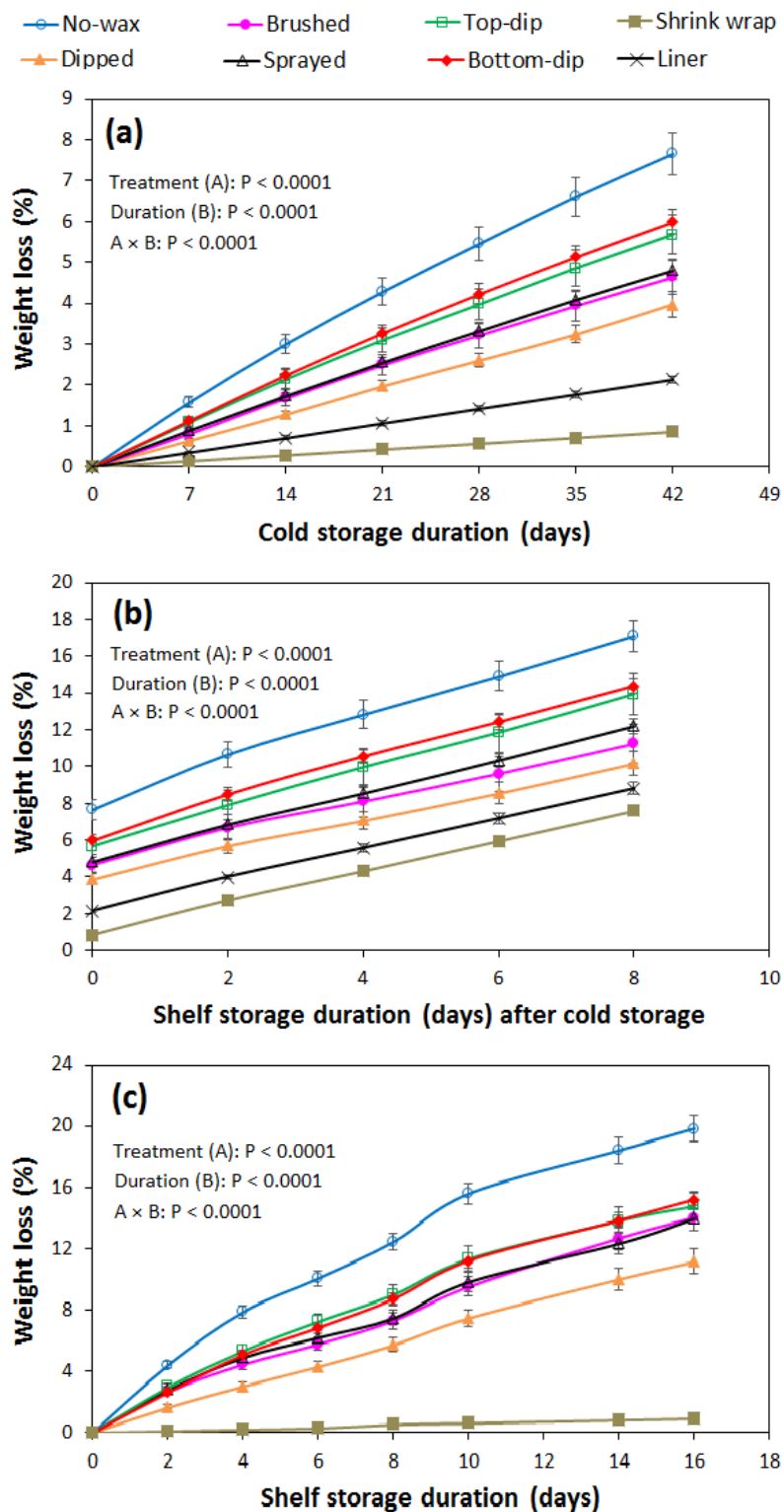


Figure 11.1 Weight loss profile of pomegranate fruit (cv. Wonderful) under different weight loss control treatments during storage: (a) at 7 °C/90 % RH for 42 d, (b) followed by additional 8 d of shelf storage at 23 °C/58 % RH and (c) under immediate prolonged shelf storage of 16d.

The data points are means ($n = 12$) and the vertical lines represent standard error of the mean. Numerical values of A and B are p-values.

11.3.2 Total colour difference (TPC) of the peel

External peel colour is a very important quality attribute of fresh fruit during marketing. It influences visual appeal and acceptance of pomegranate during marketing (Elyatem and Kader, 1984; Gil *et al.*, 1996). The overall change in peel colour was expressed as TCD (**Figure 11.2**). Generally, TCD was significantly ($P < 0.05$) influenced by the different treatments, storage duration and their interactions. TCD progressively increased with storage duration during the 42 d of cold storage and the subsequent 8 d of shelf storage. **Figure 11.2a** shows the TCD results during the additional shelf storage period, where all packaging and waxing treatments significantly minimised the overall change in colour compared to the control treatment (with no wax and packaging). At zero days of the additional shelf storage, a distinctively lower TCD is observed in the shrink wrapped and liner packaged fruit as compared to the waxed fruit. This is attributed to the generally lower moisture loss rates in the former than in the later, during the previous cold storage regime. The rapid increase in TCD for the shrink wrapped and liner packaged fruit during the additional shelf storage is because of an accelerated rate of moisture loss resulting from un-wrapping and un-packaging of fruit from the plastics to prevent induced moisture condensation inside plastic liners and accelerated fruit decay.

A similar pattern of TCD variation with treatment-duration interaction was observed for fruit under prolonged shelf storage (**Figure 11.2b**). In this case, TCD increased steadily at a higher rate for the first 8 d, followed by a progressive increase at a lower rate to the end of the 16 d. **Figure 11.3** shows that TCD is linearly related to fruit weight loss with very high positive correlations of $R^2 = 0.965$ - 0.991 during additional shelf storage and $R^2 = 0.936$ - 0.989 during prolonged shelf storage (**Table 11.1**). This suggests that TCD is a very good predictor parameter of water loss in pomegranate fruit during storage and marketing conditions. The loss of water during storage facilitates the degradation of colour pigments due to water stress (Muche *et al.*, 2018).

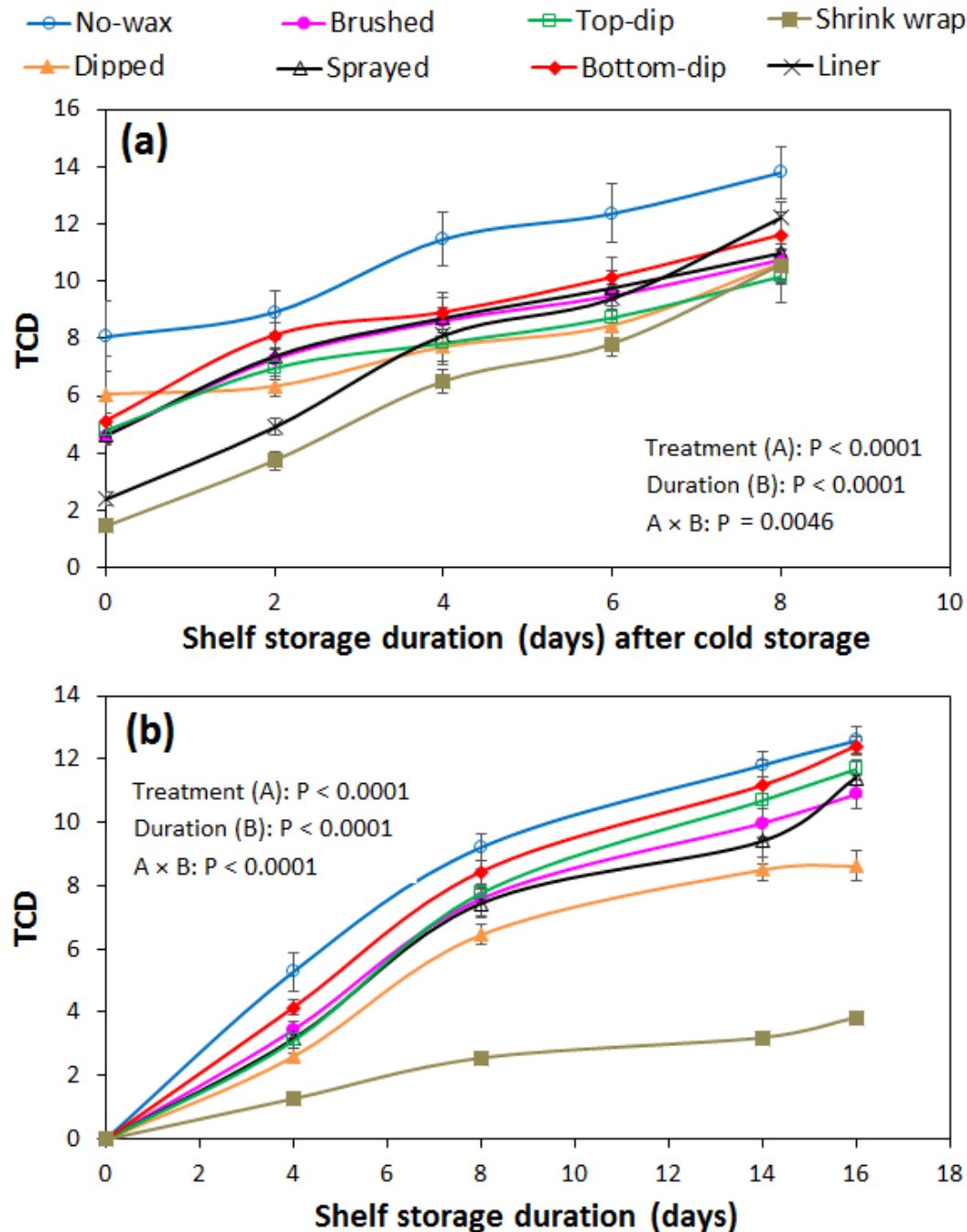


Figure 11.2 Total colour difference (TCD) profiles of pomegranate (cv. Wonderful) under different weight loss control treatments during storage: (a) the additional 8 d of shelf storage at 23 °C/58 % RH after the 42 d of cold storage at 7 °C/90 % RH and (b) under immediate prolonged shelf storage of 16 d. The data points are means ($n = 12$) and the vertical lines represent standard error of the mean. Treatments include fruit with no-package and no-wax control, fruit waxed in carnauba wax by dipping, brushing, spraying, dipping only the top half of the fruit, dipping only the bottom half of the fruit, fruit packaged by individual shrink wrapping and in plastic liners. Numerical values of A and B are p-values.

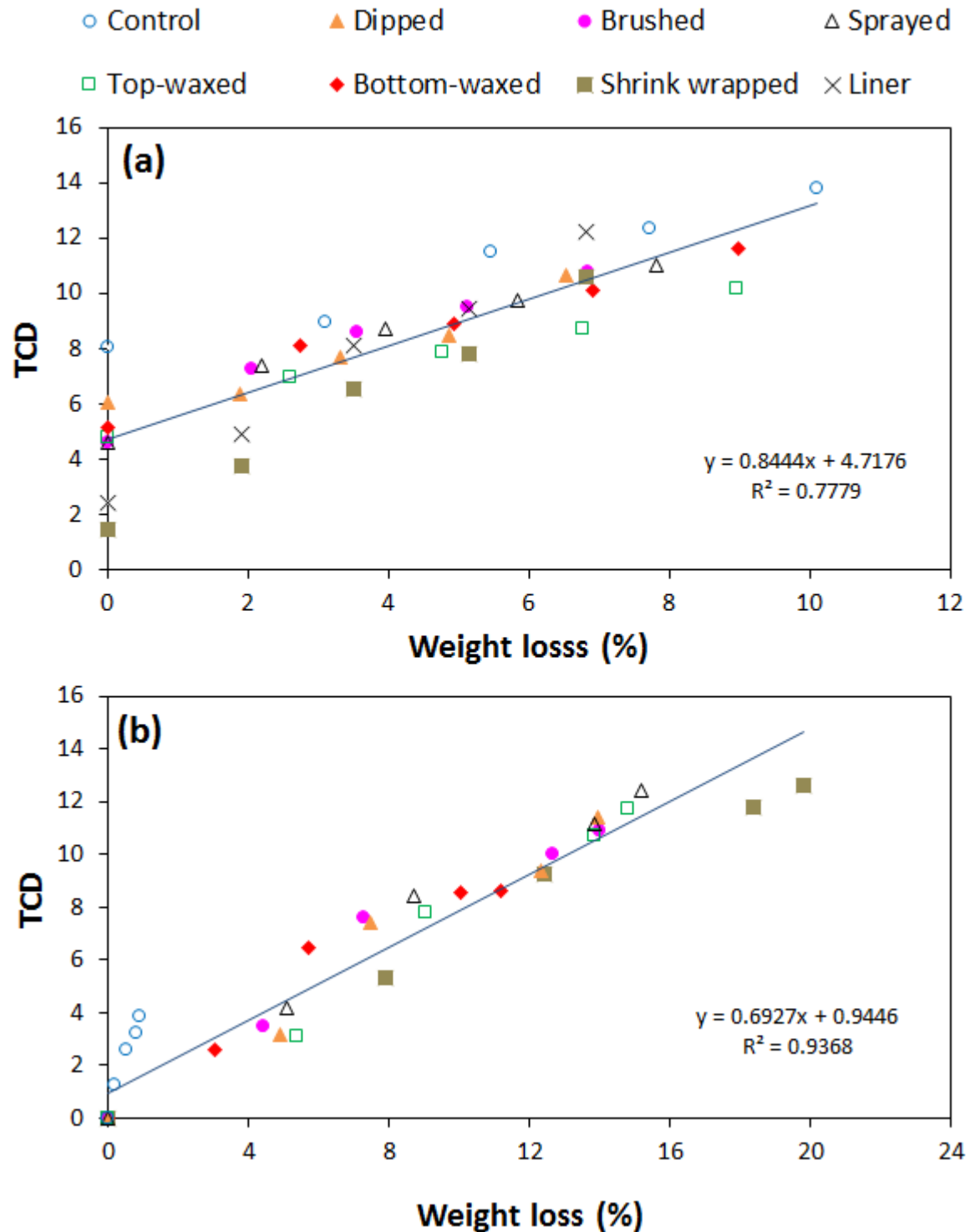


Figure 11.3 Correlations between total colour difference (TCD) and weight loss of pomegranate fruit (cv. Wonderful) under different weight loss control treatments during storage: (a) the additional 8 d of shelf storage at 23 °C/58 % RH after the 42 d of cold storage at 7 °C/90 % RH and (b) under immediate prolonged shelf storage of 16 d. A general trend line is plotted through all data points for each respective storage period.

Table 11.1 correlation coefficient (R^2) values of the linear relationship between peel total colour difference (TCD) and fruit weight loss

Treatment	42 d [7 °C] + 8 d [23 °C]	16 d [23 °C]
No-wax	0.9662	0.9886
Dipped	0.922	0.9506
Brushed	0.9684	0.9359
Sprayed	0.9648	0.9665
Top-dip	0.9817	0.9844
Bottom-dip	0.9707	0.9859
Shrink wrap	0.9913	0.9737
Liner	0.9885	
Overall	0.7779	0.9368

11.3.3 Respiration rate (RR)

Results of the respiration rate of pomegranate fruit during the 43 d of cold storage and the subsequent shelf storage of eight days are summarised in **Figures 11.4a-b**. Both the rates of oxygen consumption (R_{O_2}) and carbon dioxide production (R_{CO_2}) followed a similar trend across all tested treatments. In the study, the experimental factors of treatment type and storage duration significantly ($P < 0.05$) influenced R_{O_2} and R_{CO_2} , unlike their interactive effect. Before fruit storage, R_{O_2} and R_{CO_2} were 21.8 and 21.0 mL kg⁻¹ h⁻¹, respectively. A slight increase is observed in most treatments at the end of cold storage. At 42 d of cold storage, all waxing and packaging treatments exhibited a relatively lower R_{CO_2} than the control treatment with significantly lower results observed in the shrink wrapped and liner packaged fruit (**Figure 11.4b**). At 4 d and 8 d of subsequent shelf storage, all treatments exhibited relatively lower R_{CO_2} than the control treatment but with no significant difference.

Figure 11.5a-b shows RR of the fruit during prolonged shelf storage where R_{O_2} and R_{CO_2} decreased with storage duration. At 8 d of shelf storage, all treatments significantly had a lower R_{CO_2} than the control, except for the fruit sprayed with wax. In this case, R_{CO_2} was lowest in the shrink wrapped fruit with the value of 12.40 mL kg⁻¹ h⁻¹ compared to 16.34 mL kg⁻¹ h⁻¹ in the control fruit. Thereafter, RR did not significantly change to the end of the 16 d in all waxed and packaged fruit except for the control fruit. The tremendous decrease in R_{CO_2} observed in the control fruit at the end of the 16 d could be attributed to a change in fruit

physiology due to hardening of the rind resulting from excessive water loss. Other studies have reported a decrease in RR of pomegranate fruit with storage duration (Artés *et al.*, 1996; Nanda *et al.*, 2001; Rao, 2018). Rao (2018) observed a decreasing RR of shrink wrapped and non-wrapped pomegranate fruit (cvs. Mridula and Bhagwa) during the 28 d of shelf storage at 25–32 °C and 49–67 % RH. A similar situation was observed in a climacteric fruit (pear) stored at different temperature-relative humidity combinations (Xanthopoulos *et al.*, 2017). On the contrary, an increase of RR with storage duration has been reported in, uncoated and coated pomegranate fruit (Meighani *et al.*, 2014). The increase in other studies is attributed to tissue senescence (Sanchez-Gonzalez *et al.*, 2011).

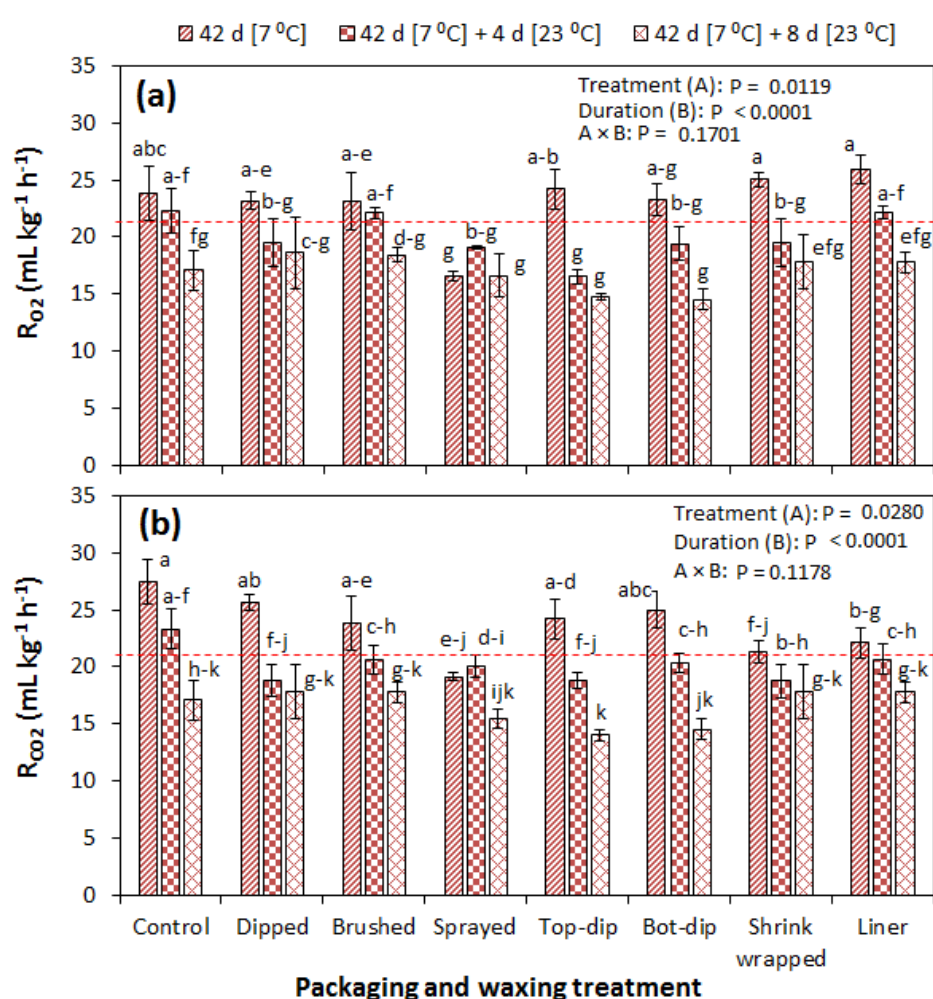


Figure 11.4 The rate of O_2 (R_{O_2}) production (a) and CO_2 (R_{CO_2}) production (b) of pomegranate fruit (cv. Wonderful) under different weight loss control treatments during storage at 7 °C/90 % RH for 42 d followed by additional 8 d of shelf storage at 23 °C/58 % RH. The bars are means ($n = 3$) and the vertical lines represent standard error of the mean. The dotted horizontal line represents values before storage. Numerical values of A and B are p-values.

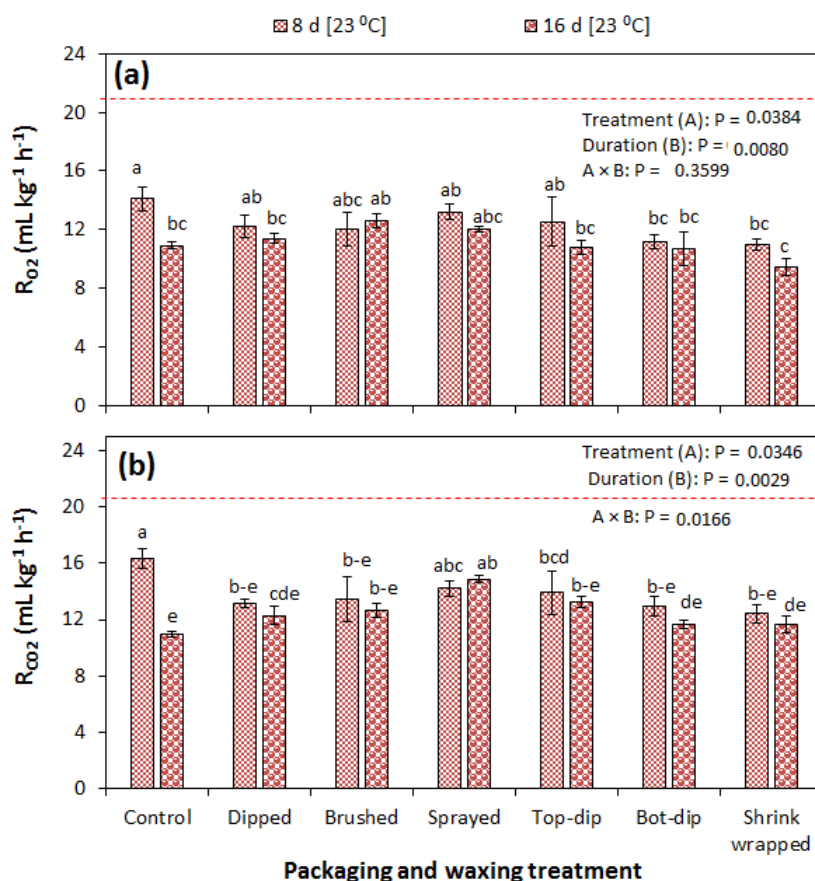


Figure 11.5 The rate of O_2 (R_{O_2}) production (a) and CO_2 (R_{CO_2}) production (b) of pomegranate fruit (cv. Wonderful) under different weight loss control treatments during prolonged shelf storage of 16 d at 23 °C/58 % RH. The bars are means ($n = 3$) and the vertical lines represent standard error of the mean. The dotted horizontal line represents values before storage. Numerical values of A and B are p-values.

11.3.4 Respiratory quotient (RQ)

The RQ is an important metabolic index in assessing characteristic respiratory kinetics of fresh produce. RQ is the ratio of CO_2 production rate of the product to the rate of O_2 consumed (Iqbal *et al.*, 2009). **Figure 11.6a-b** shows the RQ of pomegranate fruit under all tested conditions. The treatments, storage duration and their interactive effect significantly influenced RQ during the cold storage period and the subsequent shelf regime (**Figure 11.6a**). The RQ of 0.96 was observed in the control fruit before storage which significantly increased to 1.16 at 42 d of cold storage, followed by a decrease to 1.00 by the end of the 8 d subsequent shelf storage. A similar trend was observed in the bottom waxed fruit (bot-dip) and sprayed fruit. On the other hand, RQ decreased from 0.96 to 0.85 in the shrink wrapped and liner packaged fruit at the of cold storage followed by an increase to $RQ = 1$. However, treatments, storage duration and their

interactions did not have a significant influence on the RQ during the prolonged shelf storage period (**Figure 11.6b**).

Generally for all tested conditions, RQ ranged from 0.85 to 1.16 during cold storage plus shelf storage regime and 1.00 to 1.23 during prolonged shelf storage. Characteristic values of RQ are often in the range of 0.7 to 1.3 with respect to the respiratory substrate (Fonseca *et al.*, 2002). The RQ value above 1.3 is indicative of anaerobic respiration in the product (Beaudry *et al.*, 1992).

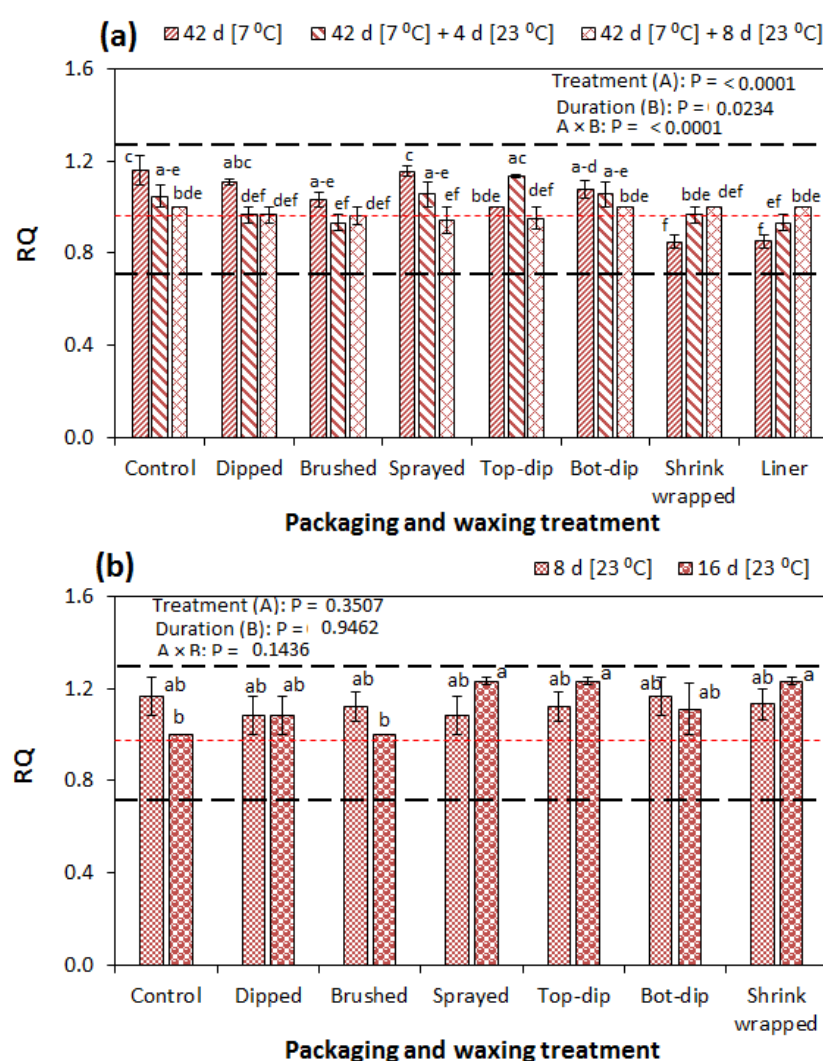


Figure 11.6 Respiratory quotient (RQ) of pomegranate fruit (cv. Wonderful) under different weight loss control treatments during storage: (a) at 7 °C/90 % RH for 42 d followed by additional 8 d of shelf storage at 23 °C/58 % RH and (b) under immediate prolonged shelf storage of 16 d. The bars are means ($n = 3$) and the vertical lines represent standard error of the mean. The dotted horizontal red line represents TA before storage, while the black dotted lines are the lower (0.7) and upper (1.3) characteristic threshold values (Fonseca *et al.*, 2002). Numerical values of A and B are p-values.

11.3.5 TSS and TA

Chemical attributes of TSS and TA are important in describing the sweetness and sourness of fruit juice taste, respectively (Tandon *et al.*, 2003; Al-Said *et al.*, 2009). The changes in the TSS of the fruit juice with storage time is presented in **Figures 11.7a-b**. The different treatments did not have a significant influence on the TSS with respect to the control treatment during the 42 d of cold storage and subsequent shelf storage (**Figures 11.7a**). Similar results of no significant difference were observed in pomegranate fruit (cv. Primosole) treated and untreated with lecithin during storage at 8 °C and 90-95 %RH for 84 d and additional 7 d of shelf storage at 20 °C and 60-65 % RH (D'Aquino *et al.*, 2012).

However, TSS varied significantly with storage conditions increasing from 16.75 °Brix before storage to 16.98 °Brix in the shrink wrapped and liner packaged fruit, 17.03-17.06 °Brix in the dipped and top-dip waxed fruit and 17.12-17.19 °Brix in the control and the rest of the wax treated fruit. This was followed by an insignificant decrease in TSS across all treatments when the fruit was transferred to shelf conditions for 8 d. The increase in TSS is attributed to the hydrolysis of starch and polysaccharides into respiration substrate sugars (Díaz-Mula *et al.*, 2009; Valero and Serrano, 2010) rather than the concentration of total solids due to moisture loss as observed in a previous study on pomegranate (cv. Hicrannar) (Selcuk and Erkan, 2014). This assumption is supported by findings from our previous study (**Chapter 4**) that water loss in different pomegranate fruit cultivars is majorly and primarily from the peel fraction rather than the arils. From that study, a weight loss of 24 % was unable to cause changes in the moisture content of the edible portion (arils) as did the un-edible portion (peels) of the three cultivars ('Acco', 'Wonderful' and 'Herskawitz') of pomegranate fruit.

On the other hand, a significantly lower TSS was observed in the shrink wrap, top-dip and brushed fruit as compared to the control fruit by the end of the 16 d of prolonged shelf storage (**Figure 11.7b**). Quite similar to our results, packaging of fruit in liners was observed to minimise the increase in TSS of pears (cv. Punjab Beauty) compared to no packaging treatment in the first 60 d of cold storage (Kaur *et al.*, 2013).

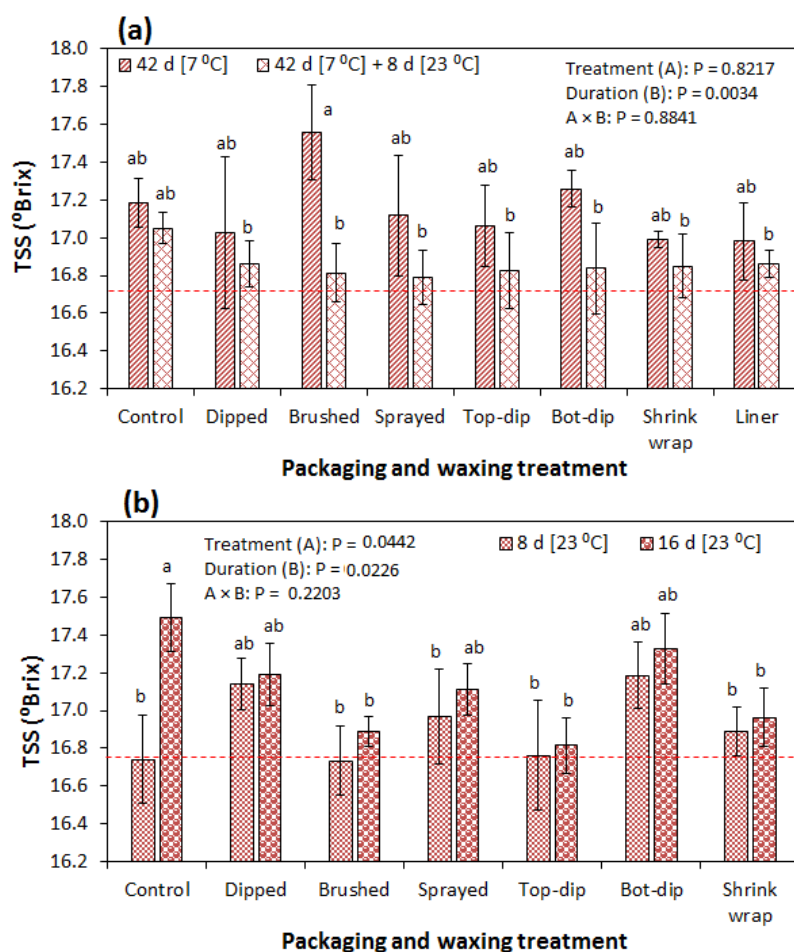


Figure 11.7 Total soluble solids (TSS) of pomegranate fruit juice (cv. Wonderful) for fruit under different weight loss control treatments during storage: (a) at 7 °C/90 % RH for 42 d followed by additional 8 d of shelf storage at 23 °C/58 % RH and (b) under immediate prolonged shelf storage of 16 d. The bars are means ($n = 12$) and the vertical lines represent standard error of the mean. The dotted horizontal line represents TSS before storage. Numerical values of A and B are p-values.

Titrateable acidity was not significantly influenced by the treatments during cold storage and the subsequent shelf storage regime (**Figure 11.8a**). However, TA significantly ($P < 0.005$) decreased with the duration from $1.62 \text{ mg } 100 \text{ mL}^{-1}$ before storage to between 0.78 and $0.92 \text{ mg } 100 \text{ mL}^{-1}$ at the end of the additional shelf storage. Comparably, a decrease in TA has been reported in different cultivars of pomegranate including ‘Wonderful’, ‘Hicrannar’ and ‘Hicaznar’ (Arendse *et al.*, 2014; Selcuk and Erkan, 2014, 2015) under different storage conditions, attributing it to the utilisation of organic acids in metabolic process. Similarly, a decrease in TA is reported for pomegranate fruit (cv. Primosole) treated and untreated with lecithin during storage at 8 °C and 90-95 % RH for 84 d and additional 7 d of shelf storage at 20 °C and 60-65 % RH (D’Aquino *et al.*, 2012). Furthermore, comparable to our results,

Mphahlele *et al.* (2016b) report a decreasing TA with no significant difference between the control and the shrink wrapped pomegranate (cv. Wonderful) fruit at the end of 90 d of storage at 7 °C and 92 % RH. However, the authors observed a significant difference in TA between the control and the liner packed fruit after the 90 d of storage. Likewise, D'Aquino *et al.* (2010) observed a significantly lower TA in the shrink wrapped fruit (cv. Primosole) than in the control fruit at the end of the 42 d at 8 °C and 85-90 % RH and additional 7 d of shelf storage at 20 °C and 65-70 % RH. In our study, TA was significantly lower in the dipped (17.14 mg 100 mL⁻¹) and shrink wrapped (16.89 mg 100 mL⁻¹) fruit than in the rest on the treatments (1.42- 1.74 mg 100 mL⁻¹) at 8 d of shelf storage (**Figure 11.8b**).

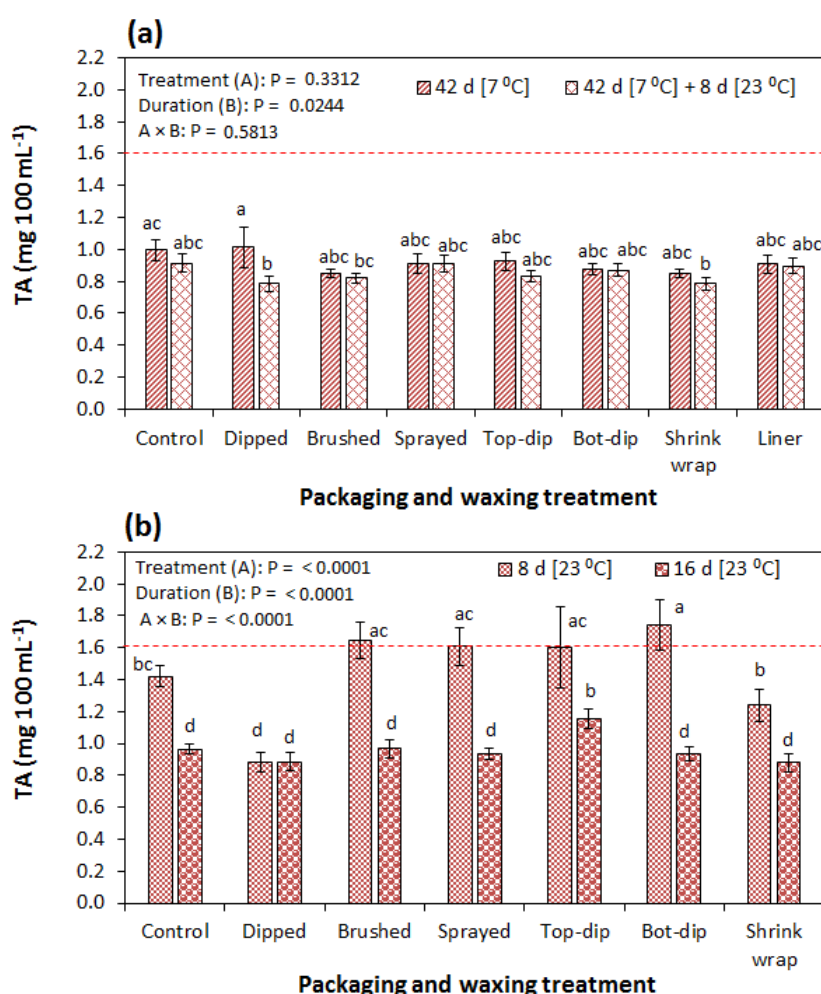


Figure 11.8 Titratable acidity (TA) of pomegranate fruit juice (cv. Wonderful) for fruit under different weight loss control treatments during storage: (a) at 7 °C/90 % RH for 42 d followed by additional 8 d of shelf storage at 23 °C/58 % RH and (b) under immediate prolonged shelf storage of 16 d. The bars are means ($n = 12$) and the vertical lines represent standard error of the mean. The dotted horizontal line represents TA before storage. Numerical values of A and B are p-values.

11.3.6 Fruit decay incidence

Results on fruit decay are presented in **Figure 11.9a-b**. Decay incidence was evaluated by visual count of all fruit with signs of decay and or mould growth. There was no visible fruit decay on fruit from all treatments except for shrink wrapped fruit. Signs of mould growth were first identified in the shrink wrapped fruit after 42 d of storage at 7 °C and 90 % RH with decay incidence of 5.6 %, followed by an increase to 11.1 % by the end of the subsequent 8 d of shelf storage at 13 °C and 58 % RH (**Figure 11.9a**). Likewise, there was no observed fruit decay in fruit from other treatments except in the shrink wrapped fruit (13.89 %) at the end of the 16 d of prolonged shelf storage (**Figure 11.9b**). Comparable to our findings, Mphahlele *et al.* (2016b) observed a decay incidence of 0.00 and 4.17 % in the liner packed, shrink wrapped pomegranate (cv. Wonderful) fruit, respectively after 30 d of cold storage at 7 °C and 92 % RH. However, the authors also observed a 4.17 % decay incidence in the control (without liner and shrink wrap) fruit. Furthermore, the authors observed an increase in decay incidence to 29.17 and 33.93, 29.17 and 16.68 % in the liner packed, shrink wrapped and control fruit at the end of the 90 d of storage. Likewise, Laribi *et al.* (1992) observed higher decay in liner packed pomegranate fruit (cv. Mollar de Elche) as compared to the control fruit with no liner.

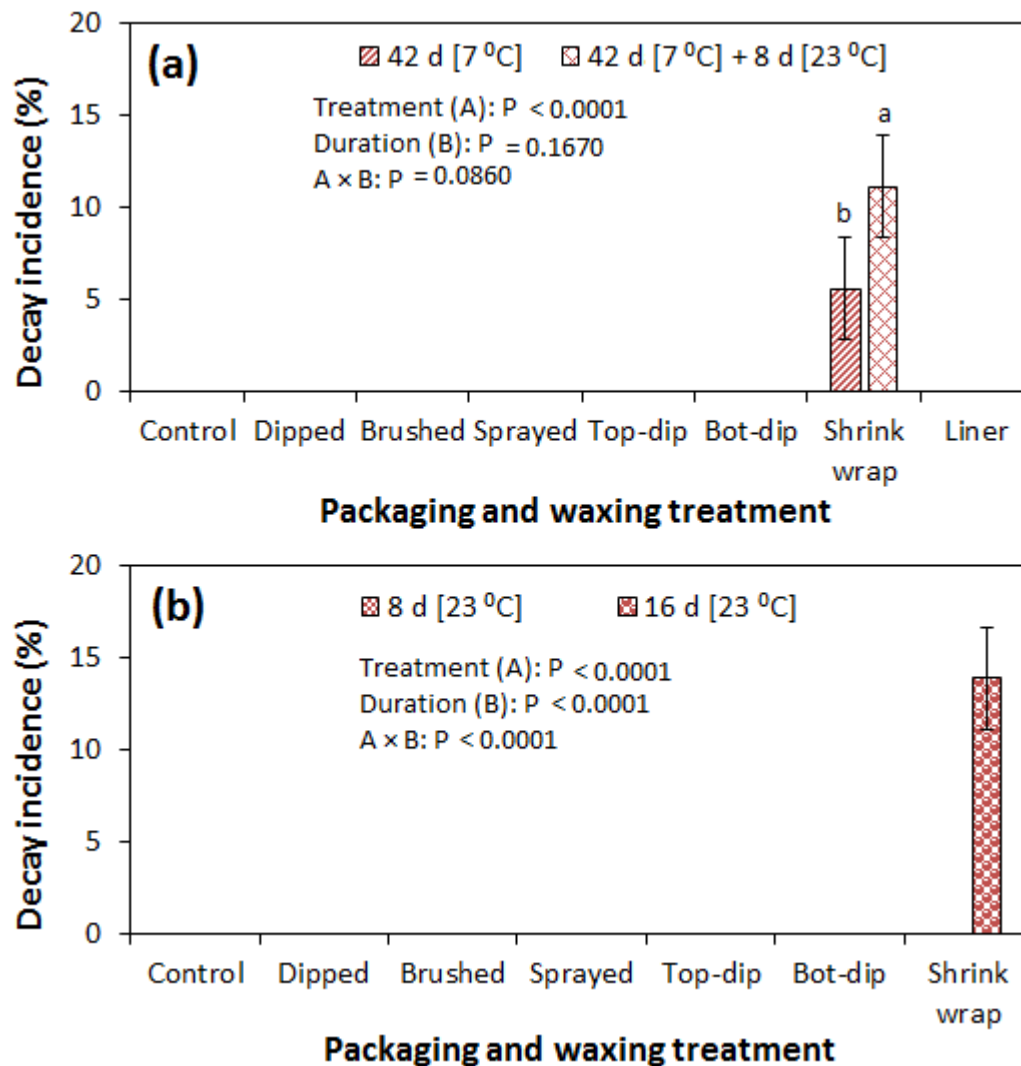


Figure 11.9 Decay incidence of pomegranate fruit (cv. Wonderful) under different weight loss control treatments during storage: (a) at 7 °C/90 % RH for 42 d followed by additional 8 d of shelf storage at 23 °C/58 % RH and (b) under immediate prolonged shelf storage of 16 d. Numerical values of A and B are p-values.

11.3.7 Sensory evaluation

Figure 11.10 shows results on the sensory evaluation carried out on the fruit arils after 42 d of cold storage and additional 8 d of shelf storage. The sweet taste attribute was scored as being much in the control fruit and moderate-much in all other treatments. The sweet taste was also scored relatively higher in the shrink wrapped, dipped and bot-dip fruit than in the top-dip. The sour taste was scored relatively lower in the liner packed and top-dip fruit than in all other treatments.

On the other hand, crispness was distinctively scored as below moderate in the control fruit and well above moderate in the shrink wrapped, liner packed, dipped, and to-dip fruit. Furthermore, only negligible (below-slight) off odour was observed in the shrink wrapped, dipped and brushed fruit. Overall, shrink wrapped, liner packed, dipped, brushed and sprayed preserved better sensory quality attributes of pomegranate arils than compared to the top-dip, bot-dip and control fruit.



Figure 11.10 Radar plot showing averaged sensory scores of pomegranate arils (cv. Wonderful) of fruit under different weight loss control treatments after 42 d of storage at 7 °C and 90 % RH followed by additional 8 d of shelf storage at 23 °C and 58 % RH. A numerical descriptive scale of 0-4 (0 = none, 1 = slight, 2 = moderate, 3 = much and 4 = very much) was used.

11.3.8 SEM

Scanning electron microscopy revealed differences in the waxing thickness and waxing patterns on the surface of the fruit (**Figure 11.10a-d**). The control sample showed the presence of widely open lenticels and micro-cracks (**Figure 11.10a**). On the other hand, waxing by dipping, brushing and spraying covers the lenticels and micro-cracks on the surface of the fruit.

The degree of wax coverage varied among the waxing methods with higher coverage (fewer gaps) observed in the dipped than in the brushed and sprayed fruit. This is attributed to the thicker wax layer in the dipped fruit ($3.86\ \mu\text{m}$) as compared to the brushed fruit ($2.89\ \mu\text{m}$) and sprayed fruit ($2.17\ \mu\text{m}$) that can easily be cracked. Quite similar results were observed in pears coated with carnauba-based wax to varying concentrations, resulting in different wax-skin coverage and thus affecting the water and gas permeance (Amarante *et al.*, 2001). These results explain why there was a relatively lower moisture loss in the dipped fruit than in the brushed fruit and sprayed fruit during the cold storage and shelf storage regimes.

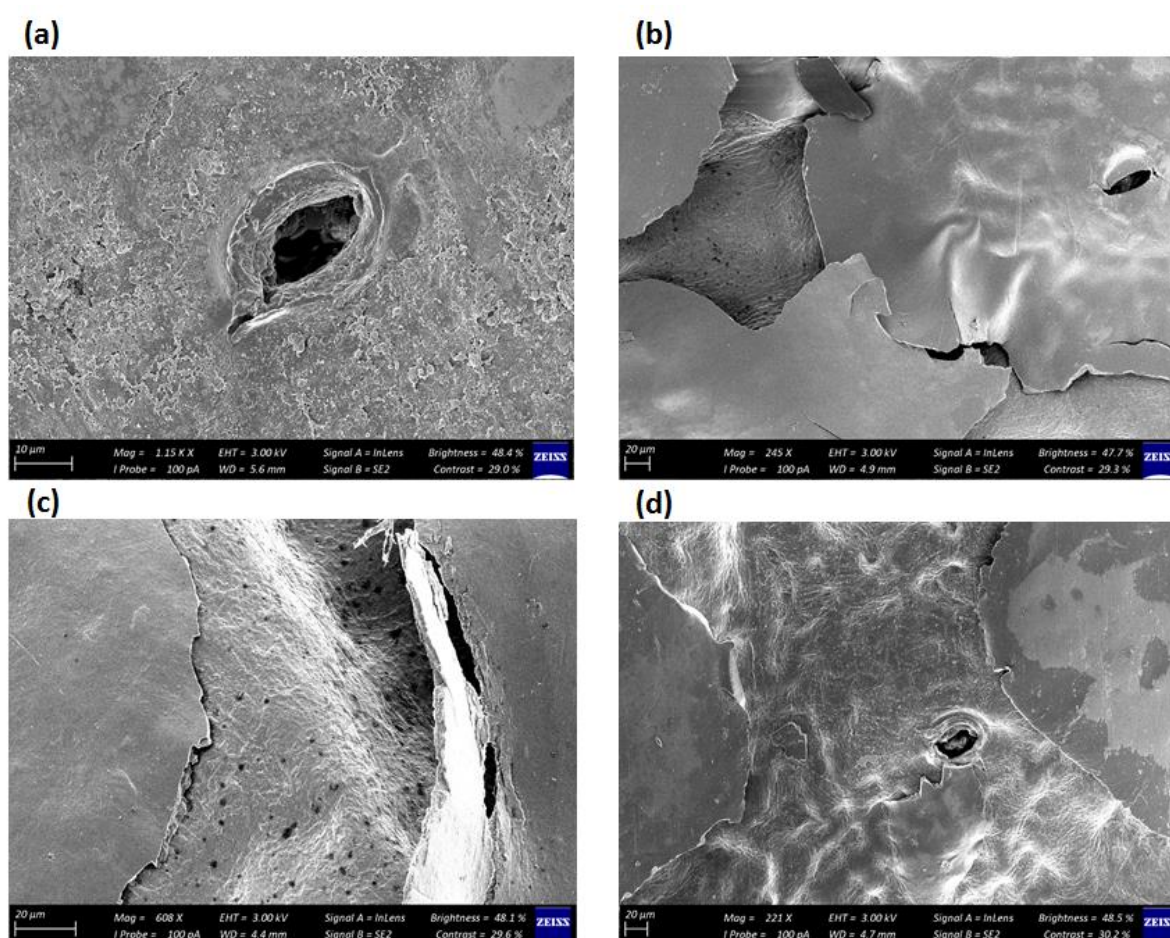


Figure 11.11 Scanning electron microscopy (SEM) images of pomegranate peel (cv. Wonderful) taken from samples of fruit: (a) with no wax, (b) dipped, (c) brushed and (d) sprayed with lac-resin based wax after they were stored for 42 d at 7 °C/90 % RH followed by additional 8 d of shelf storage at 23 °C/58 % RH.

11.4 Conclusion

This study aimed to evaluate common industrial postharvest applications in minimising water loss in pomegranate fruit during cold and shelf storage. Shrink wrapping showed the best

results in minimising water loss in pomegranate fruit (cv. Wonderful) during cold and shelf storage conditions, followed by liner packaging and wax dipping, brushing, spraying, top-dip, bottom-dip and least in the control fruit. A similar order was retained in minimising external total colour differences. Furthermore, shrink wrapping, liner packaging, dipping and brushing preserved the sensory attributes the fruit such as crispness and sweet taste. In addition, only negligible (below-slight) off odour was observed in the shrink wrapped, dipped and brushed fruit.

However, due to some visually observed decay incidence in the shrink wrapped fruit in the current study, it is, therefore, recommend that further studies consider the use of fungicide in combination with shrink wrapping to minimise water loss as well as decay incidence. Therefore, in this study liner packaging and surface waxing by dipping are considered the best options in minimising fruit water loss and preserving of postharvest quality loss. However, the current movement towards a plastic-free packaging (remove/reduce plastics) is a growing challenge in the fruit and vegetable industry. Therefore, using surface waxing should be considered as a more environmentally friendly application for minimising water loss in pomegranate fruit.

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SECTION V

General discussion integrating the results from all the previous chapters and highlights practical contribution of the studies to the pomegranate industry (Chapter 12).

CHAPTER 12

GENERAL DISCUSSION AND CONCLUSIONS

Chapter 12

12 General discussion and conclusions

1.2 Introduction

Moisture loss is one of the biggest problems faced by the pomegranate industry affecting the general postharvest quality of the fruit and resulting in financial loss. The commonly applied weight loss control technics such as surface waxing and plastic liners have created other challenges such as the production of undesirable anaerobic off flavours and moisture condensation resulting in fruit decay, respectively. Furthermore, plastic liners are at the verge of being banned by the industry to consider environmentally friendly technologies. Designing more effective and strategic control techniques require substantial scientific knowledge on the underlying mechanism of mass transport and loss. Therefore, the overall aim of the research was to investigate the mechanisms of weight loss in pomegranate fruit by investigating weight loss mechanisms, characterising associated structural and quality changes in the fruit, water transport properties of fruit tissues, developing a water transport model and its validation. Assessment of various weight loss control techniques was also carried out.

1.3 General discussion

Section I: a review of literature of the factors influencing postharvest water loss in fresh fruit, underlying mechanisms of weight loss and the modelling approach on water loss (Chapter 2 & 3).

The review of literature (**Chapter2**) identified the major pre-harvest factors relating to product type and orchard practices such as irrigation and fertilizer application; harvest factors such as applied methods and environmental conditions and postharvest factors such as pre-treatments and storage conditions. Therefore, it became clear that the irreversible weight loss during storage, distribution and retail marketing of fresh fruit is a total sum of several factors along the value chain from the orchard to retail marketing. This is relevant and informative to the industry in that there are several pre-harvest and harvest factors that are easily manipulated to greatly minimise weight loss during prolonged storage and marketing regimes. For example, cultivars that are less susceptible to moisture loss can be selected, orchard practices like irrigation schedules and harvest time of the day can be adjusted easily. By taking the necessary control precautions at pre-harvest and harvest stages, the burden of postharvest application of

loss control techniques will be minimised, improving profitability in the industry. Furthermore, the combined knowledge on pre-harvest, harvest and postharvest factors facilitate the application of a multiscale approach to minimise postharvest weight loss along the value chain.

Postharvest weight loss varies greatly among fresh produce given a multitude of factors discussed in this review. Interestingly fruit with tough thick rind such as pomegranate were more susceptible to postharvest weight loss compared to fruit with more thin and soft peel such as apples when compared at similar or optimal storage conditions (Tu *et al.*, 2000; Mukama *et al.*, 2019). However, there is limited information on the weight loss behaviour of the fruit with tough thick rinds such as pomegranates and yet it is often difficult to extrapolate control techniques from one product type to another due to significant biological and structural differences.

The review in **Chapter 3** discussed the mechanisms of weight loss in fresh fruit and the commonly applied water transport modelling approaches. Specific attention was given to the physiological mechanisms of water loss, the micro and macro structure-water loss relations, the micro, macro and multiscale approach to water loss modelling and lastly the role of imaging technologies in transport modelling. Transpiration and respiration were identified as the major processes by which fresh fruit lose weight (Elyatem and Kader, 1984; Maguire *et al.*, 2000). These processes promote depletion of water and carbohydrate substrates, respectively, causing produce weight loss through moisture and carbon dioxide diffusion and heat dissipation to the atmosphere (Gaffney *et al.*, 1985; Maguire *et al.*, 2000; Ben-Yehoshua and Rodov, 2002).

Furthermore, the review discussed the morphological and structural features of the fruit that influence water transport and weight loss, highlighting that the peel and its surface structures (stomata, lenticel and micro-cracks) play a central role in the weight loss processes. Fruit are highly heterogeneous and complex biological objects that are hierarchically structured and have features that extend from molecular scale level (such as cellulose molecule) to whole intact fruit consisting of various tissue types, which makes accurate modelling of water transport phenomena more challenging. The challenge gets bigger at industrial scale level considering tonnes of fruit in bulk storage. Therefore moisture transport in fresh fruit is modelled at different levels of spatial scales including, nano-level (e.g. Aquaporin and cell wall), micro-level (e.g. individual plant cell), meso-level (e.g. cortex tissue of an apple fruit), macro-level (e.g. whole fruit having different components) and mega level (e.g. bulk fruit in bins or pallet) (Defraeye, 2014).

Due to the high level of complexity involved, modelling of water transport in fresh fruit has been carried out in two broad approaches: the microscopic approach and the macroscopic continuum approach (Fanta *et al.*, 2013). The latter considers the specimen as a generalised homogenous entity, therefore employs parameters that are more apparent compared to physical parameters that are attainable at microscopic levels (Ho *et al.*, 2013). On the other hand, the microscopic approach considers the structural complexity of the material, recognizing the heterogeneity in transport properties existing between compartments. However, a detailed representative geometric construction is relevant. Therefore, imaging technologies such as scanning electron microscopy (SEM), x-ray computed tomography (X-ray CT) and magnetic resonance imaging (MRI) have made it possible to easily visualise the complex structures and subsequently enabled the construction of geometric models required for in-depth simulation studies.

The review highlights that a multiscale approach is one of the recent advances being applied in water transport modelling. It is a hierarchy of models describing material properties including water transport properties at different spatial scales, in such a way that the underlying sub-models are interconnected (Ho *et al.*, 2013). Multiscale combines the details obtained through microscale and mesoscale modelling and applies them at a macroscale or mega-scale level. The biggest limitation of this level of modelling is large computational time and power required. As a result, averaging procedures are applied to reduce structural details and obtain more apparent parameter estimates (Ho *et al.*, 2013).

Section II: Quantitative laboratory experiments to investigate water loss patterns in pomegranate fruit cultivars, the contribution of transpiration and respiration processes to fruit mass loss and determining water transport properties of pomegranate fruit tissues (Chapter 4-6).

The study in **Chapter 4** was aimed at characterising weight loss in pomegranate fruit based on the fundamental physical and physio-chemical attributes. Secondly, the susceptibility of pomegranate fruit cultivars to weight loss was assessed and the important question regarding to the contribution of the fruit fractions to weight loss was examined. The aim was achieved by monitoring the most important export pomegranate cultivars ('Acco', 'Herskawitz' and 'Wonderful') of South Africa, under cold storage and shelf conditions.

The major effect factors of cultivar, storage conditions and their interaction were found to significantly influence water loss and other quality attributes. The findings from the study showed that despite physiological and structural differences among pomegranate cultivars, weight loss characteristics are similar during the 42 d of cold storage. However, medium-sized fruit ('Herskawitz' and 'Wonderful') had a greater tendency towards higher weight loss and yielded significantly higher weight loss than small-sized fruit ('Acco') during prolonged storage. This is quite contrary to literature evidence that larger fruit are expected to have a lower rate of moisture loss compared to small ones, because of the higher surface area to volume ratio for the later (Ben-Yehoshua and Rodov, 2002) as discussed in details (**Chapter 2**). Therefore, the results in this particular study could be attributed to other cultivar differences such as surface stomata and lenticel density and waxy cuticle coverage.

The study further revealed that weight loss in pomegranate fruit is primarily and majorly from the peel proportion irrespective of fruit cultivar and that peel related properties such as thickness, moisture content and puncture resistance significantly influence weight loss. Generally, the findings from this study are relevant to the pomegranate industry in the following ways. This information is helpful to plant breeders in selecting against weight loss susceptible cultivars. Secondly, even though weight loss results into the deterioration of external aesthetic appeal of the fruit, the edible portion from fruit with less than 25 % weight loss retains its moisture content and can be utilised for juice extraction and processing. Furthermore, these findings suggest that studies on weight loss control and simulation can be focused on the peel to make inferences on the whole fruit. Based on the finding from this particular study (**chapter 3**), the remaining part of the research was focussed on 'Wonderful' cultivar which is one of the cultivars that are more susceptible to weight loss and but also the most important cultivated and exported among the three cultivars from South Africa. Secondly, the following sections of the research focused more on the peel which is the major source of moisture loss compared to the edible portion (arils).

The study in **Chapter 5** aimed to evaluate the transpiration and respiration characteristics of fresh produce, taking pomegranate (cv. Wonderful) fruit as a case study. The study also investigated the contribution of these processes to overall product mass loss. Furthermore, the influence of storage temperature, RH and storage time on transpiration and respiration were compared. As highlighted in the review (**Chapter 3**) that transpiration is the greatest contributing process to produce mass loss (Shirazi and Cameron, 1993) while the role

of respiration in the mass loss of fresh fruit is often considered negligible. Literature reports that in conditions of high relative humidity (RH) and temperature, respiration contributes greatly to mass loss (Gaffney *et al.*, 1985; Kang and Lee, 1998). However, a knowledge gap exists in how the process of respiration contributes to fruit mass loss. Previous studies only partly consider the contribution of respiration to fruit mass loss in the form of respiratory water loss or respiratory heat or a more generalised approach as respiratory substrate consumption (Xanthopoulos *et al.*, 2017; Bovi *et al.*, 2018). Therefore, studies that consider a broader perspective on this subject are very limited.

In this particular study the mass loss due to respiration was estimated using the stoichiometric equation of the respiration process to account for the different ways in which respiration contributes to overall mass loss: (a) respiratory carbon loss in the form of carbon dioxide gas, (b) respiratory water loss, in which the water generated by substrate oxidation becomes part of the general pool of water lost as water vapour during transpiration, and (c) the respiratory energy produced provides the latent heat necessary for more water vaporisation. Water loss and carbon loss due to the respiratory process contributed up to 35 % and 23 % at high RH (93 %) and 4 % and 3 % at lower RH (77 %) of the total mass loss, respectively. A key finding from this study is that respiration rate contributes to the total mass loss rate of fresh fruit. Unlike net transpiration rate, respiration rate contributes both to fresh mass loss (water loss) and to dry matter (carbon) loss. The specific contribution of the different respiratory mass flow components to transpiration and total mass loss are important to consider in developing predictive models and strategic control techniques, especially at high RH conditions. Furthermore, this study gives insight and promotes the quantification of fresh produce water loss with more precision. Future studies should carefully consider direct ways of quantifying real-time respiratory heat of produce and surface temperature determination. There is a need to develop predictive models that account for all the mass flow components. Therefore, the insights of this research are important in directing the improvement of existing and the design of new strategic mass loss controls in the fresh produce industry.

Chapter 6 presented the findings on water transport properties of the different tissues of pomegranate fruit (cv. Wonderful) peel. Water transport properties characterise the rate and the resistance of water movement across product tissues and membranes. Therefore, water transport properties (diffusivity and permeability) of the non-edible tissues (exocarp, mesocarp and white membrane) of pomegranate fruit under cold and shelf storage conditions, were for

the very first time determined using a diffusion cup experiment. A diffusion cup experimental setup was used to monitor moisture flux across the tissues and then moisture diffusivity (D) and permeability (K) were calculated based on Fick's law. The effect of tissue location on the fruit and determination temperature were also investigated.

The study revealed that across all tested conditions, moisture diffusivity and permeability were lower in the exocarp tissues than in the mesocarp tissues of the peel. However, removing the surface waxy layer greatly and significantly increased the transport properties of the exocarp tissue. These results demonstrate the significant role of the exocarp (outer peel) and the outermost waxy layer in minimising water loss during fruit storage. Therefore, industrial management practices such as the washing of the fruit and bulk storage into bins that compromise on the integrity of the exocarp and the waxy layer could result in excessive moisture loss during fruit storage. Very low moisture diffusivity and permeability were observed in the white membrane as compared to the exocarp and mesocarp tissues. These findings suggest the white membrane as a very protective tissue (highest resistance) against moisture loss from the edible portion (arils) of the fruit. This white membrane (inner epidermis) is the tissue that directly covers the arils (edible portion). These findings further explain why water loss in pomegranate fruit is primarily and majorly from the peel (exocarp and mesocarp) as compared to the arils (edible portion). Therefore, even though pomegranate fruit are highly susceptible to moisture loss across the numerous pores of the surface of the peel, the edible portion of the fruit is largely preserved from excessive moisture loss by the inner epidermal membrane. The differences in water transport properties among fruit tissues are attributed to huge variations in morphological and microstructural attributes across the fruit. Therefore, the micro-structural studies are recommended for a detailed understanding of mechanisms of water movement within the fruit and loss to the surrounding.

Section III: Qualitative examination of the microstructure of pomegranate peel using scanning electron microscopy, confocal laser scanning microscopy and x-ray micro-computed tomography (Chapter 7 & 8).

One of the ways of improving the accuracy and robustness of water transport models lies in capturing as many details as possible at a microscopic level rather than generalising and lumping of important parameters. This is because fruit are highly heterogeneous and complex in structure. Imaging technologies have made it possible to easily visualise the complex

structures and subsequently enabled the construction of geometric models required for in-depth simulation studies (Ho *et al.*, 2014).

Firstly, the surface structural characteristics of the peel that relate to the surrounding environment were investigated in **Chapter 7**. The aim of the study was to identify and characterise the changes in the surface structures of the pomegranate peel with respect to location on the fruit during storage. The study was carried on samples obtained from the top (calyx end), mid (equatorial region) and bottom (pedicel end) locations on randomly selected fruit. Lenticels, micro-cracks and wax layer patterns were examined under a scanning electron microscope (SEM) while the thickness of a waxy cuticle was examined under a confocal laser scanning microscope (CLSM). Results of SEM examination identified several features including surface openings such as lenticels of various shapes and micro-cracks and waxy cuticle fragmentations. The lenticels were commonly observed as either round, oval, lens-shaped or stomata-like, with the majority being stomata-like in appearance. A distinctively bright waxy cuticle was identified on the surface of the peel using CLSM. A great variation in the overall peel thickness was noticeable with respect to location on the fruit, point of measurement and storage conditions.

A higher count of lenticels, larger lenticel size, greater porosity, circularity and roundness of the individual lenticels, generally low peel thickness suggest that the pomegranate fruit was more susceptible to moisture loss at its top and mid locations as compared to the bottom location. Therefore, these findings suggest that weight loss control techniques such as surface waxing coating can be optimised through strategic application on the different locations of fruit without necessarily compromising on the aerobic respiration process of the fruit. Generally, a decreasing wax cuticle thickness profile, increased fragmentation of waxy cuticle, widening and deepening of micro-cracks, noticeable shrivelling and the general decrease in peel thickness were observed during fruit storage. This suggests that the general appearance of the fruit and the integrity of the peel were greatly compromised by end of shelf storage. Therefore, the use of surface protective coating and waxing should be considered in minimising fruit weight loss and retention of the general quality.

Secondly, **Chapter 8**, involved going deeper into the thick skin of pomegranate. This study reports the first scientific results on the changes in the 3D microstructural pore space of pomegranate peel (cv. Wonderful) due to moisture loss, using X-ray micro-computed tomography. Pore space ratio and pore characteristics such as distribution, sphericity,

compactness and surface area projection within the exocarp (outer peel) and mesocarp (inner peel) fractions were analysed. The pore space variation with respect to location (top, middle and bottom) on the fruit, peel (outer and inner peel) fractions and storage duration were specifically investigated. Findings from this study revealed that the porosity noticeably varies within the peel of an individual fruit based on a) tissue type, with higher porosity in the exocarp (external peel) than in the mesocarp (inner peel). b) The location on the fruit from which the sample is obtained. For example, in the exocarp peel fraction, porosity increased from bottom to middle to top locations, while in the mesocarp fraction more pore space was observed in the bottom than in the middle and top locations. c) Storage duration. Porosity increased with storage time as the fruit lost weight in the exocarp fraction, while a decrease was observed in the mesocarp peel fraction.

The findings from Chapter 7 and 8 of this section are relevant in developing strategic weight loss control techniques in pomegranate fruit. From these studies, we suggest that optimisation of surface coating treatments can be done by differential treatments on the top, middle and bottom of the fruit. This consideration could be a solution for minimising moisture loss without necessarily compromising on the aerobic respiration process of the fruit, consequently preserving its postharvest quality.

Section IV: Computational modelling water transport in pomegranate fruit and model validation, and the assessment of weight loss control strategies.

From a broader perspective, it is not practical to measure water loss on a very large scale such as factory level or fruit storage facility. However, through modelling, water transport can be simulated and used to develop strategic control measures on a large scale. The interaction between fluid transport and tissue microstructure is very important in the understanding of weight loss mechanisms and development of strategic weight loss control techniques. As a continuation of the work on the 3D microstructure analysis of the peel using X-ray micro-CT (**Chapter 8**), in **Chapter 9** the transport parameters describing the movement of moisture within the peel micro-structure were virtually measured by employing the Lattice-Boltzmann method (LBM). In this case, the viscous transport of water through the 3D microstructure was simulated using a direct voxel-based 2-phase material model. This procedure generated the water flow velocity field in the pore space which was then used to calculate the tortuosity and permeability of the porous peel sample and subsequently the effective moisture diffusivity

The study revealed that material properties noticeably vary within the peel based on the spatial location (top, mid and bottom) along the surface of the fruit and storage duration. Particularly, hydraulic permeability was higher in the top than in the mid and bottom samples. Furthermore, permeability increased with increasing storage time in the exocarp peel fraction. The findings of this study (in addition to finding from **Chapter 7 & 8**) further suggest that moisture loss locally varies across the surface of the pomegranate fruit with high susceptibility on the top location. Therefore, these findings are relevant in developing strategic weight loss control techniques in pomegranate fruit. For example, optimisation of surface coating treatments by differential treatments on the top, middle and bottom of the fruit will help minimise moisture loss without necessarily compromising on the aerobic respiration process and consequently preserving fruit postharvest quality.

The virtually calculated effective diffusivity values in **Chapter 9** were comparable with the experimental results ($\times 10^{-6} \text{ m}^2 \text{ s}^{-1}$) of **Chapter 6** determined using a moisture diffusion cup setup. The calculated transport parameters were then applied in the computational model development in the subsequent **Chapter (10)**. In **Chapter 10**, water transport model was developed to describe transient water transport across the whole fruit and the ultimate water loss to the surrounding environment. For the first time, the 3D water mapping within the pomegranate fruit was carried out. Simulation of water transport within intact pomegranate fruit during shelf storage conditions of 23 °C and 58 % RH were performed for the very first time. A water diffusion model based on Fick's second law was applied through a finite volume method (FVM) over the three-dimensional geometry of pomegranate fruit. The model was validated both destructively and non-destructively using gravimetric water content experiment and magnetic resonance imaging mapping, respectively. Generally, a qualitative and quantitative agreement was established between the model predictions and the gravimetric moisture concentration measurements and with the MRI measurements. The developed model is a powerful tool that can be used to study moisture loss of pomegranate fruit during storage, assessing and optimising the application of water loss control strategies. The accuracy of the model could be improved by accounting for shrinkage of fruit due to excessive moisture loss during shelf storage.

In **Chapter 11**, water loss control strategies were studied. Different applications such as liner packaging, shrink wrapping and surface waxing were evaluated. Secondly, the different methods of waxing application including dipping, brushing and spraying were investigated. Furthermore, fruit were half dipped in wax by dipping only the top or bottom half of the fruit

and this was to assess weight loss variation within individual fruit. Batch 1 fruit were stored at 7 °C and 90 % RH for 42 d and thereafter transferred to shelf conditions of 23 °C and 58 % RH for 8 d while Batch 2 fruit were immediately stored under shelf conditions for 16 d. Fruit weight loss, decay incidence, respiration rate, the external total colour difference (TCD), total soluble solids, titratable acidity and sensory quality of arils were investigated.

Results from this study revealed that shrink wrapping showed the best results in minimising water loss in pomegranate fruit (cv. Wonderful) during cold and shelf storage conditions, followed by liner packaging and wax dipping, brushing, spraying, top-dip, bottom-dip and least in the control fruit. A similar order was retained in minimising external total colour differences. Furthermore, shrink wrapping, liner packaging, dipping and brushing preserved the sensory attributes the fruit such as crispness and sweet taste. In addition, only negligible (below-slight) off odour was observed in the shrink wrapped, dipped and brushed fruit.

However, due to some visually observed decay incidence in the shrink wrapped fruit in the current study, we recommend that further studies consider the use of fungicide in combination with shrink wrapping to minimise water loss as well as decay incidence. Therefore, in this study liner packaging and surface waxing by dipping are considered the best options in minimising fruit water loss and preserving of postharvest quality loss. However, the current movement towards a plastic-free packaging (remove/reduce plastics) is a growing challenge in the fruit and vegetable industry. Therefore, using edible surface waxing should be considered as a more environmentally friendly application for minimising water loss and preserving postharvest quality of pomegranate fruit.

1.4 General conclusion and future prospects

In general, this research study has piloted the in-depth understanding of the susceptibility of pomegranate fruit to water loss. The study has established that ‘Wonderful’ and ‘Herskowitz’ cultivars are more susceptible to water loss than ‘Acco’. The thick non-edible rind is the major source of water loss compared to the edible portion (arils). The inner epidermis membrane (white membrane) covering the edible portion provides the greatest barrier against moisture loss of the arils. Furthermore, the respiration process contributes to fruit mass loss, especially under high relative humidity.

The study has established water transport properties of pomegranate tissues, which have a relevant application in industrial processing and transport simulations. Furthermore, the study

has pioneered the in-depth understanding of the microstructure of the thick pomegranate rind, exposing the associated surface structures and underneath pore space. This is vital scientific knowledge with wide industrial application in detailed modelling of mass transfer, optimisation of applications such as the use of fungicides, hot water treatments, drying kinetics, designing of film packages and optimising surface coatings.

The study has also demonstrated the use of non-invasive technology such as magnetic resonance imaging (MRI) to assess transient water profiles across intact fruit. Furthermore, the study developed and validated a water transport model that can be used in predicting and studying several postharvest applications aimed at preserving quality during storage. To improve on the accuracy of the model, future research should consider the coupling of water transport model and mechanical deformation model to account for shrinking in the fruit due to moisture loss. The study highlights surface waxing as a potential environmentally friendly solution for minimising water loss in the fruit. However, modelling of gas transport is required for accurate optimisation of surface waxing application.

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